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(54) Title: BIVALENT INHIBITOR OF FVIIa/TF/FXa COMPLEX

(57) Abstract: A novel bivalent serine protease inhibitor (I) of coagulation factor VIIa and factor Xa comprises: (i) a first serine protease inhibitor binding to factor VIIa; (ii) a linker moiety; and (iii) a second serine protease inhibitor binding to factor Xa. Also claimed are a method for inhibiting the two different serine proteases factor VIIa and factor Xa simultaneously and selectively when the two serine proteases becomes localised on the membrane protein tissue factor. The compounds and method are useful for prevention or treatment of FVIIa/TF-related diseases or disorders such as deep venous thrombosis, arterial thrombosis, post surgical thrombosis, coronary artery bypass graft (CABG), percutaneous transdermal coronary angioplastry (PTCA), stroke, tumour metastasis, inflammation, septic chock, hypotension, ARDS, pulmonary embolism, disseminated intravascular coagulation (DIC), vascular restenosis, platelet deposition, myocardial infarction, angiogenesis, or the prophylactic treatment of mammals with atherosclerotic vessels at risk for thrombosis.

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TITLE

Bivalent inhibitor of FVIIa/TF/FXa complex.

FIELD OF INVENTION

This invention relates to novel compounds which bind to and inhibit the enzymatic activity associated with plasma coagulation factor VIIa and factor Xa. Specifically, the invention provides examples of molecules having selective inhibition effect of coagulation factor VIIa and factor Xa when located on tissue factor. The two serine protease inhibitors of the hybrid molecule of the present invention are linked via a linker moiety. The invention also relates to pharmaceutical compositions comprising the novel compounds as well as their use in treatment of or prophylaxis of thrombotic or coagulopathic related diseases or disorders including vascular diseases and inflammatory responses.

BACKGROUND OF INVENTION

Blood coagulation is a process consisting of a complex interaction of various blood components, or factors, which eventually gives rise to a fibrin clot. Generally, the blood components which participate in what has been referred to as the coagulation "cascade" are proenzymes or zymogens, enzymatically inactive proteins, which are converted to proteolytic enzymes by the action of an activator, itself an activated clotting factor. Coagulation factors that have undergone such a conversion and generally referred to as "active factors", and are designated by the addition of the letter "a" to the name of the coagulation factor (e.g. FVIIa).

In order to form a fibrin clot and thereby stop a bleeding, activated factor X (FXa) is required. Activated factor X (FXa) is needed to convert prothrombin to thrombin, which then converts fibrinogen to fibrin as a final stage in forming a fibrin clot. There are two systems, or pathways that promote the activation of factor X. The "intrinsic pathway" refers to those reactions that lead to thrombin formation through utilisation of factors present only in plasma. A series of protease-mediated activations ultimately generates factor IXa, which, in conjunction with factor VIIIa, cleaves factor X into Xa. FVIIa and its cofactor tissue factor

(TF) in the "extrinsic pathway" of blood coagulation effect an identical proteolysis. While the relative importance of the two coagulation pathways in haemostasis is unclear, FVIIa and TF have in recent years been found to play a pivotal role in the initiation and regulation of blood coagulation.

Factor VIIa (FVIIa) is a two-chain, 50 kilodalton (kDa) vitamin-K dependent, plasma serine protease which participates in the complex regulation of in vivo haemostasis. Factor VIIa is generated from proteolysis of a single peptide bond from its single chain zymogen, Factor VII, which is present at approximately 0.5 μg/ml in plasma. The zymogen is catalytically inactive (Williams et al., J. Biol. Chem. 264:7536-7543 (1989); Rao et al., Proc. Natl. Acad. Sci. USA. 85:6687-6691 (1988)). The conversion of zymogen Factor VII into the activated two-chain molecule occurs by cleavage of an internal peptide bond. In human factor VII, the cleavage site is at Arg152-Ile153 (Hagen et al., Proc. Natl. Acad. Sci. USA 83: 2412-2416 (1986); Thim et al., Biochemistry 27:7785-7793 (1988)).

Like several other plasma proteins involved in haemostasis, FVII is dependent on vitamin K for its activity. Vitamin K is required for the gamma-carboxylation of multiple glutamic acid residues that are clustered in the amino terminus of the protein. These gamma-carboxylated glutamic acids are required for the metal-associated interaction of FVII with phospholipids. Thus, in the presence of calcium ions, Factor VIIa binds with high affinity to exposed tissue factor (TF), an integral membrane protein. TF acts as a cofactor for Factor VIIa, enhancing the proteolytic activation of its substrates Factors VII (FVII), Factor IX (FIX) and Factor X (FX).

TF is a 263 amino acid residue glycoprotein composed of a 219 residue extracellular domain, a single transmembrane domain, and a short cytoplasmic domain. TF is a membrane bound protein and does not normally circulate in plasma. The binding of FVIIa is mediated entirely by the extracellular domain of TF, which has been extensively characterised.

TF is expressed constitutively on cells separated from plasma by the vascular endothelium. Its expression on endothelial cells and monocytes is induced by exposure to inflammatory cytokines or bacterial lipo-polysaccharides. Upon tissue injury or vessel disruption, the exposed extracellular domain of TF in the presence of Ca^{2+} and phospholipid forms a high affinity calcium-dependent complex with FVII (Nemerson and Gentry, Biochemistry 25:4020-

4033 (1986)). Once bound to TF, FVII can be activated by peptide bond cleavage to yield serine protease FVIIa. The enzyme that catalyses this step *in vivo* is believed to be FXa. *In vitro* FXa, thrombin, FVIIa/TF and FIXa can catalyse this cleavage. FVIIa has only weak activity upon its physiological substrates FX and FIX whereas the FVIIa/TF complex rapidly activates FX and FIX. The FVIIa/TF complex constitutes the primary initiator of the extrinsic pathway of blood coagulation. The complex initiates the extrinsic pathway by activation of FX to Factor Xa (FXa), FIX to Factor IXa (FIXa), and additional FVII to FVIIa. The action of FVIIa/TF leads ultimately to the conversion of prothrombin to thrombin, which carries out many biological functions. Among the most important functions of thrombin is the conversion of fibrinogen to fibrin, which polymerises to form a clot. The FVIIa/TF complex also participates as a secondary factor in extending the physiological effects of the contact activation system.

The binding of FVIIa to TF is tight with a dissociation constant in the picomolar range. The binding is essential for full proteolytic activity of FVIIa and co-localisation of FVIIa on the cell surface together with FX (Martin et al., FASEB J. 9: 852-859 (1995)). FX is a macromolecular substrate for the FVIIa/TF complex, a reaction that is strongly membrane-dependent. The binding of FX to FVIIa/TF has been determined from the K_m-value (analogous to the dissociation constant) and have been shown to be about 20 nM (Gertz et al., Circulation. 98:580-587 (1998)). This specific binding results in a transient complex between TF, FVIIa and FXa (FVIIa/TF/FXa), where FVIIa and FXa are closely assembled on the extracellular domain of TF. The activated FXa then leaves the FVIIa/TF complex and converts prothrombin to thrombin leading to platelet activation (Monroe et al., Br. J. Haematol. 88: 364-371 (1994)).

The involvement of this plasma protease system has been suggested to play a significant role in a variety of clinical manifestations including arterial and venous thrombosis, septic chock, adult respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC) and various other disease states such as restenosis, atherosclerosis and tumour metastasis.

The presence in serum of an endogenous inhibitor of FVIIa/TF has long been recognised. Following initiation of the coagulation cascade, FVIIa/TF is down-regulated by tissue factor pathway inhibitor (TFPI), a feedback inhibitor that prevents further activation of zymogen substrates.

It is often desirable to selectively block or inhibit the coagulation cascade in a patient, for example during kidney dialysis, or to treat deep vein thrombosis, DIC, atherosclerosis and a host of other medical disorders. Typically, anticoagulants such as heparin, coumarin, derivatives of coumarin, indandione derivatives, thrombin inhibitors, or factor Xa inhibitors have been used. For example, heparin treatment or extracorporal treatment with citrate ions (U.S. Patent 4, 500, 309) may be used in dialysis to prevent coagulation during the course of treatment. Heparin is also used in preventing deep vein thrombosis in patients undergoing surgery. Treatment with heparin and other anticoagulants may, however, have undesirable side effects. Available anticoagulants generally act throughout the body, rather than acting specifically at the site of injury, i. e., the site at which the coagulation cascade is active. Heparin, for example, may cause severe bleedings. Furthermore, with a half-life of approximately 80 minutes, heparin is rapidly cleared from the blood, necessitating frequent administrating. Because heparin acts as a cofactor for antithrombin III (AT III), and AT III is rapidly depleted in DIC treatment, it is often difficult to maintain the proper heparin dosage, necessitating continuous monitoring of AT III and heparin levels. Heparin is also ineffective if AT III depletion is extreme. Further, prolonged use of heparin may also increase platelet aggregation and reduce platelet count, and has been implicated in the development of osteoporosis. Indandione derivatives may also have toxic side effects.

Other known anticoagulants comprise thrombin and factor Xa inhibitors derived from bloodsucking organisms. Antithrombins, hirudin, hirulog and hirugen are recombinant proteins or peptides derived from the leach Hirudo medicinalis, whereas the factor Xa inhibitor antistatin and the recombinant derivative rTAP are tick-derived proteins. Inhibitors of platelet aggregation, such as monoclonal antibodies or synthetic peptides, which interfere with the platelet receptor GPIIb/IIIa are also effective as anticoagulants. Tsuda, Y; Biochemistry 33(48): 14443-14451 (1994) and Maraganore, J M; Biochemistry 29(30): 7095-7101 (1990) both describes peptide inhibitors of thrombin based on hirudin sequence (hirulogs).

Bleeding complications are observed as an undesired major disadvantage of anti-thrombin, anti-factor Xa, as well as anti-platelet reagents. This side effect is strongly decreased or absent with inhibitors of the FVIIa/TF activity. Such anticoagulants comprise the physiological inhibitor TFPI (tissue factor pathway inhibitor) and modified FVII (FVIIai), which is FVIIa modified in such a way that it is catalytically inactive and thus not able to catalyse

the conversion of FX to FXa, but still able to bind to TF in competition with active endogenous FVIIa.

In addition to the anticoagulants briefly described above, several naturally occurring proteins have been found to have anticoagulant activity. For example, Reutelingsperger (U.S. Patent No. 4,736, 018) isolated anticoagulant proteins from bovine aorta and human umbilical vein arteries. Maki et al. (U.S. Patent No. 4, 732, 891) discloses human placenta-derived anticoagulant proteins. In addition, AT III has been proposed as a therapeutic anticoagulant (Schipper et al., Lancet 1 (8069): 854-856 (1978); Jordan, U.S. Patent No. 4, 386, 025; Bock et al., U.S. Patent No. 4, 517, 294).

For long term prophylactic treatment and increased compliance it would be desirable to have access to low-molecular-weight compounds which, apart from intravenous administration, also allows for administration via a route other than intravenously and which have an inhibitory effect on the extrinsic TF activity similar to that of FVIIai.

Publications describing low-molecular-weight compounds which down-regulate FVIIa/TF activity include JP 07242538 which describes naphthalene derivatives with tissue factor antagonist activity; US 5639739 which describes FVII-inhibiting peptide analogues derived from imidazolyl-boronic acid; JP 6157591 which describes compounds based on peptides from TFPI; WO 90/03390, WO 95/00541, WO 96/18653, and EP 500 800 which describe peptides derived from FVIIa having FVIIa/TF antagonist activity.

Furthermore, WO 98/03632 describes bivalent agonists having affinity for one or more G-coupled receptors, and Burgess, L.E. et al., Proc. Natl. Acad. Sci. USA 96, 8348-8352 (July 1999) describes "Potent selective non-peptidic inhibitors of human lung tryptase".

There is still a need in the art for improved compositions having anticoagulant activity at relatively low doses and which does not produce any undesirable side effects. There is also a need for compositions which allows for non-intraveneous administration, for example orally, at relatively low doses and which does not produce any undesirable side effects. The present invention provides low molecular weight anticoagulants that act specifically on the TF initiated process at sites of injury.

Citation or identification of any reference in the above section of this application shall not be construed as an admission that such reference is available as prior art to the present application.

SUMMMARY OF THE INVENTION

The present invention relates to a bivalent inhibitor which inhibits the protease activity of FVIIa and FXa when associating with tissue factor in the FVIIa/TF/FXa complex initiating the extrinsic coagulation cascade. The bivalent inhibitor consists of a serine protease inhibitor (SP inhibitor) of FVIIa (including a moiety or binding motif that binds to and inhibits FVIIa) and a serine protease inhibitor of FXa (including a moiety or binding motif that binds to and inhibits FXa) linked by a suitable linking moiety (LM), said LM being covalently linked to the binding motifs. Compared to inhibitors targeting only one of these serine proteases, a bivalent protease inhibitors provide additional advantages in terms of higher binding affinity (potency), as well as enhanced specificity against similar cellular host enzymes for reduced toxicity effects.

It is an object of the present invention to provide compounds having pharmacological activity as inhibitors of FVIIa/TF/FXa activity, and thus to provide methods for inhibiting TF-mediated coagulation activity.

The present invention thus provides novel bivalent SP having the formula A-(LM)-D that binds via FVIIa and FXa to a complex comprising TF, FVIIa and FXa, wherein A is a moiety that binds FVIIa; D is a moiety that binds FXa; and LM is a linker moiety, and wherein the affinity of binding of said compound to said complex is at least about 10-fold higher than the affinity of binding of said compound to either free FVIIa or free FXa.

The compounds are useful for the prevention or treatment of FVIIa/TF-related diseases or disorders including vascular diseases and inflammatory responses.

The invention also provides a method for reducing TF-mediated coagulation activity, said method comprising contacting a FVIIa/TF/FXa complex with a compound having the formula A-(LM)-D, wherein A is a moiety that binds FVIIa; D is a moiety that binds FXa; and LM is a linker moiety, and wherein the affinity of binding of said compound to said complex is at least about 10-fold higher than the affinity of binding of said compound to either free FVIIa or free

FXa.

In one embodiment of the invention, the moiety A binds to the active site of FVIIa.
In another embodiment, the moiety D binds to the active site of FXa.
In yet another embodiment, A binds to the active site of FVIIa and D binds to the active site of FXa.

In one embodiment, the compound A-(LM)-D co-localises the FVIIa and FXa on TF in such a way that the distance measured between the γ-oxygen atoms of the serine residue 195 in the active site of FVIIa and FXa, respectively, is at least about 24 angstroms.
In another embodiment, the compound A-(LM)-D co-localises the FVIIa and FXa on TF in such a way that the distance measured between the γ-oxygen atoms of the serine residue 195 in the active site of FVIIa and FXa, respectively, is at least about 24 angstroms and at most about 58 angstroms.

In one embodiment, the LM is a peptide or LM is selected among the following structures: C_{1-18} alkyl, a 1 to 18 -membered straight or branched chain comprising carbon and at least one N, O or S atom in the chain, C_{3-8}cycloalkyl, a 3 to 8 -membered cyclic ring comprising carbon and at least one N, O or S atom in the ring, aryl, C_{1-4}alkyl-substituted aryl, heteroaryl, C_{1-4} alkyl-substituted heteroaryl; the structures optionally substituted with one or more of the following groups: hydroxy, oxo, amino, C_{1-4} monoalkylamino, C_{1-4} dialkylamino, acylamino, sulfonyl, carboxy, carboxamido , halogeno, C_{1-6} alkoxy, C_{1-6} alkylthio, trifluoroalkoxy, alkoxy-carbonyl, haloalkyl.

In one embodiment, the reduction of coagulation activity is a reduction of proteolytic activity of either or both of FVIIa and FXa.
In another embodiment, the reduction of coagulation activity is a reduction of proteolytic activity of FVIIa. In another embodiment, the reduction of coagulation activity is a reduction of proteolytic activity of FXa.

The invention also provides pharmaceutical compositions for inhibiting TF-mediated coagulation activity, comprising an amount of the compound of claims 1-11 and a pharmaceutically acceptable carrier or excipient.

In one embodiment, the composition further contains a platelet aggregation inhibitor.

The invention also provides the use of a compound as defined in claims 1-11 for the manufacture of a medicament for preventing or treating FVIIa/TF related diseases or disorders; and a method for preventing or treating FVIIa/TF related diseases or disorders in a mammal by administering an an effective amount of at least one compound as defined in claim 1-11 to the mammal.

The mammal is preferably a human.

Further objects will become apparent from the following description.

LIST OF FIGURES

Figure 1 shows a schematic outline of selected enzymes and mediators that modulate the coagulation, contact, fibrinolytic, inflammatory, and complement pathways. Activation of these pathways can lead to the clinical states indicated.

Figure 2 schematically shows the bivalent inhibitor of the invention.

Figure 3 shows inhibition of FXa selectively on the FVIIa/TF complex.

Figure 4 shows specific inhibition of FXa on the FVIIa/TF complex.

Figure 5 shows time dependency of FXa inhibition on the FVIIa/TF complex.

Figure 6 shows concentration dependency of FXa inhibition on the FVIIa/TF complex.

Figure 7 shows the optimal covalent-linked bivalent SP inhibitor for the FVIIa/TF/FXa complex.

DETAILED DESCRIPTION OF THE INVENTION

It is extremely difficult to design protease inhibitors that are specific enough to be used clinically without affecting other systems depending on proteases. If other systems are affected side effects are to be expected. This becomes even more unfortunate in prophylactic treatment where the drug is supposed to be used daily for extended periods of time. A small inhibitory effect on another protease will suffice to cause side effects.

The present invention provides selective inhibitors of TF-mediated coagulation activity.

Following tissue or vessel disruption, the extrinsic coagulation cascade are driven by the formation of a transient complex in which FVIIa and FXa are bound to exposed TF. In this complex the active sites of FVIIa and FXa must become localised on the membrane surface in an arranged manner. From the X-ray structure of the FVIIa/TF complex (Banner et al., Nature 380: 41-46 (1996)) and fluorescence studies (McCallum et al., J. Biol. Chem. 271: 28168-28175 (1996); McCallum et al., J. Biol. Chem. 272: 30160-30166 (1997)) it is shown that the active site of FVIIa is localised approximately 80 Å above the membrane surface.

In order for FVIIa to proteolytically activate FX, the protease domains of these two coagulation factors must be in physical contact with each other. This need for close proximity or physical contact limits the localisation of the active sites of FVIIa and FXa to an extremely small region just above the surface of the membrane. Sufficiently small compounds possessing two binding motifs, i. e., compounds according to the present invention, will bind FVIIa and FXa simultaneously when FVIIa and FXa are located on TF.

A bivalent serine protease (SP) inhibitor directed simultaneously against FVIIa and FXa when bound to exposed TF has now been provided.

In order to achieve multiple binding, proper design of the bivalent SP inhibitor against the two different sites in FVIIa and FXa is needed. The functional affinity of such a ligand is dependent both on the overall concentration but particularly on the localisation of the molecule (i.e., the binding of one inhibitor motif to the first enzyme) which favours the binding of the second motif on a bivalent inhibitor to the second enzyme. After the binding of one motif, the diffusion of its tethered partner will be constrained within a radius equal to the length of the spacer connecting the two molecules. This constraining effect will increase the local concentration for the motif binding to the second site.

It is important that the bivalent SP inhibitor according to the invention neither inhibits the free form of FVIIa or of FXa, especially not FXa. This is important in order to avoid bleeding problems such as those seen when interfering with the intrinsic pathway. This is achieved by using SP inhibitors that are only active in relatively high molar range against the free form of FVIIa and FXa, i.e. above the therapeutic concentration for the bivalent inhibitor.

The binding of the bivalent SP inhibitor toward the FVIIa/TF/FXa complex will be of significant higher affinity. This is because of the avidity effect achieved in the bivalent binding mode. A consequence of this is that inhibition of free FVIIa can be prevented; only the FVIIa molecules that are bound to TF will be shut off; the same applies to FXa . The strength in the bivalent binding (the avidity) creates a therapeutic window wherein the bivalent SP inhibitor, at a specific concentration, is active against the FVIIa/TF/FXa complex but not active against the free forms of FVIIa and FXa (or other proteases circulating in the blood).

In other words, the bivalent SP inhibitors are designed from inhibitors active against free FVIIa or free FXa or other proteases. The dosage used (of the bivalent SP inhibitor) allows only inhibition of FVIIa and FXa assembled on TF and does not inhibit the free forms of FVIIa or FXa or other proteases.

The bivalent SP inhibitors of the present invention thus binds to FVIIa and FXa co-locallised on TF with an affinity at least about 10-fold higher than the affinity of binding of said compound to either free FVIIa or free FXa. In one embodiment, the affinity is at least about 20-fold higher than the affinity of binding of said compound to either free FVIIa or free FXa; in another embodiment, the affinity is about 30-fold higher; in yet another embodiment, the affinity is about 50-fold higher; in yet another embodiment, the affinity is about 100-fold higher than the affinity of binding of said compound to either free FVIIa or free FXa.

A bivalent SP inhibitor contains three distinct motifs that can be modulated in order to achieve preferential inhibiting characteristics: the two SP inhibitors, and the linker moiety (LM) or backbone joining the inhibitors. The LM should not be regarded only as a spacer that connects the two SP inhibitors. It is possible to utilise the LM for optimising the binding properties of the bivalent SP inhibitor in order to obtain the highest affinity and selectivity for the FVIIa/TF/FXa complex possible. An undersized LM will prevent binding to the two sites simultaneously and too long a LM means too much loss in entropy in binding to the second binding site, thereby reducing functional affinity. In addition, the chemical property of the LM can also be varied in order to achieve good solubility, especially in cases where the inhibitors themselves have poor solubility and good bioavailability.

The Examples illustrating the invention demonstrate that low-molecular bivalent SP inhibitors targeted to FVIIa and FXa simultaneously have extraordinary selective inhibition when these two coagulation factors are bound in complex with the membrane bound glycoprotein TF.

This is the first demonstration to the inventors knowledge that a low-molecular entirely synthetic compound can specifically inhibit FVIIa and FXa when they are located on TF. In the disclosed Examples the affinity is approximately 20-fold higher in the presence of TF compared to when TF is absent, this being mediated by a bivalent binding mode. Bivalent binding increases avidity, which compensate for weak binding, but we also show from the disclosed Examples that the selectivity increase enormously. This increase in selectivity is not accomplished by the inhibitor *per se*; it is due to the specific macromolecular binding of FXa to the FVIIa/TF complex. This specific binding of FXa to the FVIIa/TF complex is essential for a selective FXa inhibition on top of selectivity the SP inhibitor posses. In addition, the results below show a very high inhibition-dependency of the length of the LM or backbone connecting the two SP inhibitors. The optimal length of the bivalent SP inhibitor [SP inhibitor-(LM)-SP inhibitor] range from 6 to 40 Å (defined as the distance between the primary α-amine in the two FFR moieties used in the Examples) or 24 to 58 Å (defined as the distance between the γ-oxygen atoms of the serine residues 195 in the active sites of FVIIa and FXa, respectively).

The bivalent SP inhibitors according to the invention may bind to the active sites of FVIIa and FXa; or they may bind to the active site of FVIIa and a domain of FXa which is able to bind to the moiety; or they may bind to the active site of FXa and a domain of FVIIa which is able to bind to the moiety; or they may bind to a domain of FVIIa and a domain of FXa which are able to bind to the moieties. Preferred are bivalent SP inhibitors binding to at least one active site; most preferred are bivalent SP inhibitors binding to the active sites of both FVIIa and FXa. In one embodiment, the K_i-value of each binding motif (or each monovalent inhibitor) is about 50 μM or less; in another embodiment, the K_i-value is about 30 μM or less; in another embodiment, the K_i-value is about 20 μM or less, in yet another embodiment, the K_i-value is about 10 μM or less; in yet another embodiment, the K_i-value is about 5 μM or less; and in yet another embodiment, the K_i-value is about 1 μM or less.

Schematically, this is represented in Figure 2 in which the serine protease inhibitors are depicted as blocks (A) and (D), being connected together by the linker moiety (LM) depicted as a rod.

Abbreviations

Abbreviations used throughout the description include:

TF	tissue factor
FVIIa, fVIIa	factor VIIa, activated factor VII
FIXa, fIXa	factor IXa, activated factor IX
FXa, fXa	factor Xa, activated factor X
FVII, fVII	zymogen (single-strand, non-activated) factor VII
FIX, fIX	zymogen (single-strand, non-activated) factor IX
FX, fX	zymogen (single-strand, non-activated) factor X
FVIIa/FVII	FVIIa and/or FVII
FIXa/FIX	FIXa and/or FIX
FXa/FX	FXa and/or FX
FVIIa/TF	complex between tissue factor and Factor VIIa
FVIIa/TF/FXa, FVIIa/TF/FX	complex formed by factor VIIa, tissue factor and factor X, transformed into a transient complex between factor VIIa, tissue factor and factor Xa
PT	Prothrombin time
APTT	Activated partial thromboplastin time
SP	serine protease
FFR	Phe-Phe-Arg or D-Phe-Phe-Arg
D-FFR	D-Phe-Phe-Arg
dim-FFR	dimeric form of FFR, two FFRs bound together by linking moiety
dim-FFR-FVIIa	dim-FFR bound to factor VIIa, preferably in active site
FFR-cmk, FFR-CMK	Phe-Phe-Arg chloromethylketone or D-Phe-Phe-Arg chloromethylketone
D-FFR-cmk, D-FFR-CMK	D-Phe-Phe-Arg chloromethylketone
EGR	Glu-Gly-Arg or D-Glu-Gly-Arg
D-EGR	D-Glu-Gly-Arg
EGR-cmk, EGR-CMK	Glu-Gly-Arg chloromethylketone or D-Glu-Gly-Arg chloromethylketone
K_i	dissociation constant, inhibition constant of enzyme-inhibitor complex

It is to be understood that when the designation "D" immediately precedes a letter abreviation for an amino acid as defined above, that amino acid is the non-natural d-enantiomer.

A FVIIa/TF mediated or associated process or event, or a process or event associated with TF-mediated coagulation activity, is any event, which requires the presence of FVIIa/TF. Such processes or events include, but are not limited to, formation of fibrin which leads to thrombus formation; platelet deposition; proliferation of smooth muscle cells (SMCs) in the vessel wall, such as, for example, in intimal hyperplasia or restenosis, which is thought to result from a complex interaction of biological processes including platelet deposition and thrombus formation, release of chemotactic and mitogenic factors, and the migration and proliferation of vascular smooth muscle cells into the intima of an arterial segment; and deleterious events associated with post-ischemic reperfusion, such as, for example, in patients with acute myocardial infarction undergoing coronary thrombolysis.

The no-reflow phenomenon, that is, lack of uniform perfusion to the microvasculature of a previously ischemic tissue has been described for the first time by Krug et al., (Circ. Res. 1966; 19:57-62).

The general mechanism of blood clot formation is reviewed by Ganong, in Review of Medical Physiology, 13th ed., Lange, Los Altos Calif., pp 411-414 (1987). Coagulation requires the confluence of two processes, the production of thrombin which induces platelet aggregation and the formation of fribrin which renders the platelet plug stable. As outlined in Fig. 1, the process comprises several stages each requiring the presence of discrete proenzymes and profactors. The process ends in fibrin crosslinking and thrombus formation. Fibrinogen is converted to fibrin by the action of thrombin. Thrombin, in turn, is formed by the proteolytic cleavage of prothrombin. This proteolysis is effected by FXa which binds to the surface of activated platelets and in the presence of FVa and calcium, cleaves prothrombin. FVIIa/TF is required for the proteolytic activation of FX by the extrinsic pathway of coagulation. Therefore, a process mediated by or associated with FVIIa/TF, or an TF-mediated coagulation activity includes any step in the coagulation cascade from the formation of the FVIIa/TF complex to the formation of a fibrin platelet clot and which initially requires the presence of FVIIa/TF. For example, the FVIIa/TF complex initiates the extrinsic pathway by activation of FX to FXa, FIX to FIXa, and additional FVII to FVIIa. FVIIa/TF mediated or associated process, or TF-mediated coagulation activity can be conveniently measured employing standard assays such as those described in Roy, S., (1991) J. Biol. Chem. 266:4665-4668, and O'Brien, D. et al., (1988) J. Clin. Invest. 82:206-212 for the conversion of FX to FXa in the presence of FVIIa/TF and other necessary reagents.

"Diseases or disorders related to TF-mediated coagulation activity" or "FVIIa/TF-related diseases or disorders", or "thrombotic or coagulopathic related diseases or disorders" include, but are not limited to, inflammatory responses and chronic thromboembolic diseases or disorders associated with fibrin formation, including vascular disorders such as deep venous thrombosis, arterial thrombosis, post surgical thrombosis, coronary artery bypass graft (CABG), percutaneous transdermal coronary angioplastry (PTCA), stroke, tumour metastasis, angiogenesis, thrombolysis, arteriosclerosis and restenosis following angioplastry, acute and chronic indications such as inflammation, septic chock, septicemia, hypotension, adult respiratory distress syndrome (ARDS), disseminated intravascular coagulopathy (DIC), pulmonary embolism, platelet deposition, myocardial infarction, or the prophylactic treatment of mammals with atherosclerotic vessels at risk for thrombosis, and other diseases. The

FVIIa/TF related disorder is not limited to in vivo coagulopatic disorders such as those named above but includes ex vivo FVIIa/TF related processes such as coagulation that may result from the extracorporeal circulation of blood, including blood removed in-line from a patient in such processes as dialysis procedures, blood filtration, or blood bypass during surgery.

The term "free FVIIa" means FVIIa molecules that are not bound to TF but are allowed to diffuse freely in the blood.

The term "free FXa" means FXa molecules that are not bound to the FVIIa/TF complex but are allowed to diffuse freely in the blood.

The term "FVIIa activity" means the amidolytic/proteolytic activity associated with the active site of FVIIa.

The term "FXa activity" means the amidolytic/proteolytic activity associated with the active site of FXa.

The term "TF-mediated coagulation activity" means coagulation initiated by TF through the formation of the FVIIa/TF complex and its activation of FIX and FX to FIXa and FXa, respectively.

The term "active site" and the like when used herein with reference to FVIIa refer to the catalytic and zymogen substrate binding site, including the "S_1" site of FVIIa as that term is defined by Schecter, I. and Berger, A., (1967) Biochem. Biophys. Res. Commun. 7:157-162.

The term "active site" and the like when used herein with reference to FXa refer to the catalytic and zymogen substrate binding site, including the "S_1" site of FXa as that term is defined by Schecter, I. and Berger, A., (1967) Biochem. Biophys. Res. Commun. 7:157-162.

By "relipidated TF" is meant full-length (263 amino acids) reconstituted into membranes containing a mixture of phosphatidylcholine and phosphatidylserine lipids.

By "FVIIa/TF/FXa complex" is meant the complex formation between TF and the coagulation factors FVIIa and FXa co-localised on tissue factor.

By "co-localising" is meant placing the FVIIa and FXa on TF in a suitable way for formation of the FVIIa/TF/FXa complex.

By "inhibitors of FVIIa/TF/FXa " is meant any substance where the affinity of binding of said substance to said complex is at least about 10-fold higher than the affinity of binding of the substance to either free FVIIa or free FXa.

By "K_i" is meant the dissociation constant (also called inhibition constant) of an enzyme-inhibitor complex. For a description of the kinetics of inhibition of enzymes and the Michaelis-Menten equation, see, for example, Stryer, L., Biochemistry, Freeman and Company, 1981, pp. 116-120; or Lehninger, Principles of Biochemistry, Worth Publishers, Inc, 1982, pp. 207-243.

By "binding motifs or domains" is meant domains of a larger molecule, said domains having an affinity to FVIIa or FXa, as well as smaller compounds having an affinity to FVIIa or FXa. The binding motifs may be isolated from a larger molecule, such as for example a naturally occurring peptide or protein; or they may be compounds known *per se* as SP inhibitors. The binding motifs may be linked together by a synthesised or naturally occurring linker moiety, or they may both be located in one naturally occurring molecule, such as for example a naturally occurring peptide or other type of compound, one part of the molecule showing inhibitory action against FVIIa and another part of the molecule showing inhibitory action against FXa, the compound thereby being a bivalent inhibitor.
The bivalent SP inhibitor of the invention may also be synthesised from a binding motif being a compound in itself known as, or found to be, a FVIIa inhibitor and another binding motif being a compound in itself known as, or found to be, a FXa inhibitor, the two compounds linked together by a suitable linking moiety, and thus becoming a bivalent inhibitor.

The binding motifs or domains of the bivalent SP inhibitors are bonded covalently to a molecular linker moiety. The LM allows the two binding motifs or domains to bind to the two serine proteases simultaneously. To accomplish this, the backbone must be long enough to span the FVIIa/TF/FXa complex, but flexible enough to permit binding to a second serine

protease. In other words, suitable backbones are those that can assume a stable, extended secondary structure configuration, while remaining flexible and sufficiently soluble in aqueous or physiological systems.

By "linker moiety" (LM) or "backbone" is meant any biocompatible molecule functioning as a means to link the two binding motifs or domains. Each motif or domain is linked to the molecular LM via a covalent bond, preferably via an amide or peptide bond between an amino group of the LM and a carboxyl group, or its equivalent, of the binding motif or domain, or vice versa. By "flexible" is meant that the LM comprises a plurality of carbon-carbon σ bonds having free rotation about their axes, so as to allow the two binding motifs or domains to be separated by a distance suitable to bind to FVIIa and FXa when located on TF.

The LM is first chosen among structures that in combination with the A and D binding motifs or domains achieve a distance of 24 to 58 Å between the γO atoms of the serine residue 195 in the active site of FVIIa and FXa when the bivalent SP inhibitor is bound to FVIIa/TF/FXa complex. This distance includes both the LM and the two SP inhibitors (A and D). The length of the linker itself is therefore dependent upon the structural character, or size, of the two SP inhibitors A and D.

One skilled in the art will recognise that various combinations of atoms provide for variable chain length molecules based upon known distances between various bonds (Morrison and Boyd, Organic Chemistry, 3rd ed , Allyn and Bacon, Inc., Boston, Mass. (1977)).

Suitable LMs, or backbones, comprise group(s) such as, but are not limited to, peptides; polynucleotides; sacharides including monosaccharides, di- and oligosaccharides, cyclodextrins and dextran; polymers including polyethylene glycol, polypropylene glycol, polyvinyl alcohol, hydrocarbons, polyacrylates and amino-, hydroxy-, thio- or carboxy-functionalised silicones, other biocompatible material units; and combinations thereof. Such LM materials described above are widely commercially available or obtainable via synthetic organic methods commonly known to those skilled in the art.

The LM may, for example, be selected among the following structures:
C_{1-18}alkyl, a C_{1-18} alkyl chain comprising at least one N, O or S atom in the chain, C_{3-8}cycloalkyl, a C_{3-8}cycloalkyl ring comprising at least one N, O or S atom in the ring, aryl, C_{1-8}alkyl-substituted aryl, heteroaryl, C_{1-8} alkyl-substituted heteroaryl, a peptide or a peptidomimetic; the structures optionally substituted with one or more of the following groups: hydroxy, oxo, amino, C_{1-8} alkyl, C_{1-4} monoalkylamino, C_{1-4} dialkylamino, acylamino, sulfonyl,

carboxy, carboxamido , halogeno, C_{1-6} alkoxy, C_{1-6} alkylthio, trifluoroalkoxy, alkoxycarbonyl, haloalkyl . The LM may be straight chained or branched and may contain one or more double or triple bonds. The LM may contain one or more heteroatoms like N,O or S.

It should be noted that peptides and proteins as described herein can comprise, and amino acids as used herein, refer not only to "natural", i.e., naturally occurring amino acids, but also to "non.classical" D-amino acids including, but not limited to, the D-isomers of the common amino acids, α-isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogues in general. In addition, the amino acids can include Abu, 2-amino butyric acid; γ-Abu, 4-aminobutyric acid; ε-Ahx, 6-aminohexanoic acid; Aib, 2-amino-isobutyric acid; β-Ala, 3-aminopropionic acid; Orn, ornithine; Hyp, trans-hydroxyproline; Nle, norleucine; Nva, norvaline.

By "combinations thereof" is meant that the LM can comprise more than one class of the groups described above, as well as being able to comprise more than one member within a class. Where the LM comprises more than one class of group, such LM is preferably obtained by joining different units via their functional groups. Methods for forming such bonds involve standard organic synthesis and are well known to those of ordinary skill in the art.

In order to select a suitable linker moiety, the LM is coupled to different SP inhibitors (A and D) and screened in the below FVIIa and FXa amidolytic assay. LM is characterised as suitable when the FVIIa and FXa inhibition is enhanced 10-fold or more in any combination of (A) and (D) in the presence of an equivalent amount of relipidated TF compared to when no relipidated TF is present.

The LM can comprise functional groups, such as, for example hydroxy, oxo, amino, C_{1-4} monoalkylamino, acylamino, sulfonyl, carboxy, carboxamido , halogeno, C_{1-6} alkoxy, C_{1-6} alkylthio, trifluoroalkoxy, alkoxycarbonyl, or haloalkyl groups. The LM can also comprise charged functional groups, such as for example, ammonium groups or carboxylate groups. The charged functional groups can provide bivalent SP inhibitors with sufficient solubility in aqueous or physiological systems, provide reactive sites for ionic bonding with other species, and enhance their avidity to FVIIa/TF/FXa complex. It is within the purview of one of skill in

the art to select a particular acid, and concentration thereof, to confer optimal solubility and avidity properties to the bivalent SP inhibitors. Preferably, the total amount of charged functional groups are minimised so as to maximise the bivalent SP inhibitors' specificity for FVIIa/TF/FXa complex, but not so as to significantly decrease solubility.

The binding motifs or domains are situated on the LM such that the distance between them is sufficient to allow the motifs or domains to bind to FVIIa and FXa when situated on exposed TF. Preferably, the distance of LM in combination with the A and D binding motifs or domains achieve a distance of 24 to 58 Å between the γO atoms of the serine residue 195 in the active site of FVIIa and FXa when the bivalent SP inhibitor is bound to FVIIa/TF/FXa complex. This distance includes both the LM and the two SP inhibitors (A and D). While the binding motifs or domains can be situated anywhere on the LM as long as the distance between them suffices (branched LM), each motif or domain is preferably located at each terminal end of a linear LM.

It is to be understood that the above mentioned distance of about 24 to about 58 Angstroms is the distance which separates the active sites of FVIIa and FXa when situated on exposed TF. Because the LM of the bivalent SP inhibitor is flexible, the bivalent SP inhibitor is capable of assuming a conformation that allows the binding motifs or domains thereof to bind to FVIIa and FXa in FVIIa/TF/FXa. Thus, for complete and effective binding of the motifs or domains to FVIIa and FXa, the motifs or domains should be able to assume a distance of about 24 to about 58 Angstroms therebetween. Such a distance can be measured, or predicted theoretically, by any method known in the art. For example, molecular modelling can be used to determine distances between binding motifs or domains in bivalent SP inhibitors of the invention, based upon, e.g., the predicted conformation of the molecule. Molecular modelling programs that can be used are commonly known and available in the art. Alternatively, the distance between binding motifs or domains is measured by reacting a bivalent SP inhibitor, preferably a bivalent SP inhibitor having amino functional groups, with a reactive species, such as, for example, a halogen-substituted benzoyl halide, known to convert a non-crystalline species to a crystalline species. Such a crystalline species can be subjected to x-ray diffraction, such that the distance between its binding motifs or domains can be determined.

It is to be understood that the two binding motifs or domains in a bivalent SP inhibitor can be the same or different.

By a "FVIIa inhibitor" is meant a substance binding to FVIIa and decreasing or preventing the FVIIa-catalysed conversion of FX to FXa. A FVIIa inhibitor may be identified as a substance A, which reduces the amidolytic activity by at least 50% at a concentration of substance A at 400 μM in the FVIIa amidolytic assay described by Persson et al. (Persson et al., J. Biol. Chem. 272: 19919-19924 (1997)). Preferred are substances reducing the amidolytic activity by at least 50% at a concentration of substance A at 300 μM; more preferred are substances reducing the amidolytic activity by at least 50% at a concentration of substance A at 200 μM.

By a "FXa inhibitor" is meant a substance binding to FXa and decreasing or preventing the FXa-catalysed conversion of prothrombin to thrombin. A FXa inhibitor may be identified as a substance D, which reduces the amidolytic activity by at least 50% at a concentration of substance D at 400 μM in the FXa amidolytic assay described by Soerensen et al. (Sørensen et al., J. Biol. Chem. 272: 11863-11868 (1997)). Preferred are substances reducing the amidolytic activity by at least 50% at a concentration of substance D at 300 μM; more preferred are substances reducing the amidolytic activity by at least 50% at a concentration of substance D at 200 μM.

The "FVIIa inhibitor" and "FXa inhibitor" may be selected from any one of several groups of FVIIa- or FXa directed inhibitors. Such inhibitors are broadly categorised for the purpose of the present invention into i) inhibitors which reversibly bind to FVIIa (FXa, respectively) and are cleavable by FVIIa (FXa, respectively), ii) inhibitors which reversibly bind to FVIIa (FXa, respectively) but cannot be cleaved, and iii) inhibitors which irreversibly bind to FVIIa (FXa, respectively). For a review of inhibitors of serine proteases see Proteinase Inhibitors (Research Monographs in cell and Tissue Physiology; v. 12) Elsevier Science Publishing Co., Inc., New York (1990).

The inhibitor moiety of the present invention may also be an irreversible FVIIa- or FXa serine protease inhibitor. Such irreversible active site inhibitors generally form covalent bonds with the protease active site. Such irreversible inhibitors include, but are not limited to, general serine protease inhibitors such as peptide chloromethylketones (see, Williams et al., J. Biol. Chem. 264:7536-7540 (1989)) or peptidyl cloromethanes; azapeptides; acylating agents

such as various guanidinobenzoate derivatives and the 3-alkoxy-4-chloroisocoumarins; sulphonyl fluorides such as phenylmethylsulphonylfluoride (PMSF); diisopropylfluorophosphate (DFP); tosylpropylchloromethyl ketone (TPCK); tosyllysylchloromethyl ketone (TLCK); nitrophenylsulphonates and related compounds; heterocyclic protease inhibitors such as isocoumarines, and coumarins.

Examples of peptidic irreversible inhibitors of FVIIa and FXa include, but are not limited to, Phe-Phe-Arg chloromethylketone, D-Phe-Phe-Arg chloromethylketone, dansyl-Phe-Phe-Arg chloromethylketone, dansyl-D-Phe-Phe-Arg chloromethylketone, Phe-Pro-Arg chloromethylketone, D-Phe-Pro-Arg chloromethylketone, dansyl-Phe-Pro-Arg chloromethylketone, dansyl-D-Phe-Pro-Arg chloromethylketone, L- og D-Glu-Gly-Arg chloromethylketone.

Examples of FVIIa inhibitors also include benzoxazinones or heterocyclic analogues thereof such as described in PCT/DK99/00138.

Examples of other inhibitors of FVIIa and FXa include, but are not limited to, small peptides such as for example Phe-Phe-Arg, D-Phe-Phe-Arg, dansyl-Phe-Phe-Arg, dansyl-D-Phe-Phe-Arg, Phe-Pro-Arg, D-Phe-Pro-Arg, dansyl-Phe-Pro-Arg, dansyl-D-Phe-Pro-Arg, L- and D-Glu-Gly-Arg; peptidomimetics; benzamidine systems; heterocyclic structures substituted with one or more amidino groups; aromatic or heteroaromatic systems substituted with one or more C(=NH)NHR groups in which R is H, C_{1-3}alkyl, OH or a group which is easily split of in vivo.
Bivalent SP inhibitors comprising reversible inhibitors are generally preferred.

"Treatment" means the administration of an effective amount of a therapeutically active compound of the invention with the purpose of preventing any symptoms or disease state to develop or with the purpose of curing or easing such symptoms or disease states already developed. The term "treatment" is thus meant to include prophylactic treatment.

By "inhibitors of FVIIa/TF/FXa activity" is meant compounds that inhibit FVIIa/TF/FXa in *in vitro* assays of amidolytic and proteolytic activity (see, for example, Persson, E et al., J. Biol. Chem., 272(32), 19919-19924 (1997) and Sørensen, B.B. et al., J. Biol. Chem., 272(18), 11863-11868 (1997)) and thus are able to prolong the TF-induced coagulation in human plasma. They do so by preventing the activated Factor X (FXa) from leaving the

FVIIa/TF/FXa complex formed and thus prevent the FXa-catalysed conversion of prothrombin into thrombin.

By "modulators of the TF pathway" is meant compounds that modulate the coagulation process through an inhibitory action on the FVIIa/TF/FXa complex. By this action, the initiation and acceleration of the blood coagulation cascade upon exposure of TF to flowing blood is prevented.

"Modulating and normalising an impaired haemostatic balance" means achieving an effect on the coagulation system measurable in vitro assays and/or animal models which diminishes the risk for thrombosis or bleedings.

As used herein:

The terms "$C_{1-n'}$-alkyl" wherein n' can be from 2 through 18, as used herein, alone or in combination, refers to a straight or branched, saturated or unsaturated hydrocarbon chain having from one to the specified number of carbon atoms. An olefinically unsaturated branched or straight group having from 2 to the specified number of carbon atoms and at least one double bond is also referred to as alkenyl; an unsaturated branched or straight group having from 2 to the specified number of carbon atoms and at least one triple bond is also referred to as alkynyl. The C_{1-18}-alkyl residues include aliphatic hydrocarbon residues, unsaturated aliphatic hydrocarbon residues, alicyclic hydrocarbon residues. Examples of the aliphatic hydrocarbon residues include saturated aliphatic hydrocarbon residues having 1 to 8 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec.butyl, tert.butyl, n-pentyl, isopentyl, neopentyl, tert.pentyl, n-hexyl, isohexyl. Example of the unsaturated aliphatic hydrocarbon residues include those having 2 to 6 carbon atoms such as ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 2-methyl-1-propenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 3-methyl-2-butenyl, 1-hexenyl, 3-hexenyl, 2,4-hexadienyl, 5-hexenyl, ethynyl, 1-propionyl, 2-propionyl, 1-butynyl, 2-butynyl, 3-butynyl, 1-pentynyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexynyl, 3-hexynyl, 2,4-hexadiynyl, 5-hexynyl.

The term C_{3-6}-cycloalkyl means an alicyclic hydrocarbon residue including saturated alicyclic hydrocarbon residues having 3 to 6 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl; and C_{5-6} unsaturated alicyclic hydrocarbon residues having 5 to 6 carbon at-

oms such as 1-cyclopentenyl, 2-cyclopentenyl, 3-cyclopentenyl, 1-cyclohexenyl, 2-cyclohexenyl, 3-cyclohexenyl.

The term "C_{1-6}-alkoxy" as used herein, alone or in combination, refers to a straight or branched monovalent substituent comprising a C_{1-6}-alkyl group linked through an ether oxygen having its free valence bond from the ether oxygen and having 1 to 6 carbon atoms e.g. methoxy, ethoxy, propoxy, isopropoxy, butoxy, pentoxy.

The term "C_{1-6}-alkylthio" as used herein, alone or in combination, refers to a straight or branched monovalent substituent comprising a C_{1-6}-alkyl group linked through an thioether sulfur atom having its free valence bond from the thioether sulfur and having 1 to 6 carbon atoms.

The terms "aryl" and "heteroaryl" as used herein refers to an aryl which can be optionally substituted or a heteroaryl which can be optionally substituted and includes phenyl, biphenyl, indene, fluorene, naphthyl (1-naphthyl, 2-naphthyl), anthracene (1-anthracenyl, 2-anthracenyl, 3-anthracenyl), thiophene (2-thienyl, 3-thienyl), furyl (2-furyl, 3-furyl) , indolyl, oxadiazolyl, isoxazolyl, quinazolin, fluorenyl, xanthenyl, , isoindanyl, benzhydryl, acridinyl, thiazolyl, pyrrolyl (2-pyrrolyl), pyrazolyl (3-pyrazolyl), imidazolyl (1-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), triazolyl (1,2,3-triazol-1-yl, 1,2,3-triazol-2-yl 1,2,3-triazol-4-yl, 1,2,4-triazol-3-yl), oxazolyl (2-oxazolyl, 4-oxazolyl, 5-oxazolyl), thiazolyl (2-thiazolyl, 4-thiazolyl, 5-thiazolyl), pyridyl (2-pyridyl, 3-pyridyl, 4-pyridyl), pyrimidinyl (2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl), pyrazinyl, pyridazinyl (3- pyridazinyl, 4-pyridazinyl, 5-pyridazinyl), quinolyl (2-quinolyl, 3-quinolyl, 4-quinolyl, 5-quinolyl, 6-quinolyl, 7-quinolyl, 8-quinolyl), isoquinolyl (1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 5-isoquinolyl, 6-isoquinolyl, 7-isoquinolyl, 8-isoquinolyl), benzo[b]furanyl (2-benzo[b]furanyl, 3-benzo[b]furanyl, 4-benzo[b]furanyl, 5-benzo[b]furanyl, 6-benzo[b]furanyl, 7-benzo[b]furanyl), 2,3-dihydro-benzo[b]furanyl (2-(2,3-dihydro-benzo[b]furanyl), 3-(2,3-dihydro-benzo[b]furanyl), 4-(2,3-dihydro-benzo[b]furanyl), 5-(2,3-dihydro-benzo[b]furanyl), 6-(2,3-dihydro-benzo[b]furanyl), 7-(2,3-dihydro-benzo[b]furanyl), benzo[b]thiophenyl (2-benzo[b]thiophenyl, 3-benzo[b]thiophenyl, 4-benzo[b]thiophenyl, 5-benzo[b]thiophenyl, 6-benzo[b]thiophenyl, 7-benzo[b]thiophenyl), 2,3-dihydro-benzo[b]thiophenyl (2-(2,3-dihydro-benzo[b]thiophenyl), 3-(2,3-dihydro-benzo[b]thiophenyl), 4-(2,3-dihydro-benzo[b]thiophenyl), 5-(2,3-dihydro-benzo[b]thiophenyl), 6-(2,3-dihydro-benzo[b]thiophenyl), 7-(2,3-dihydro-benzo[b]thiophenyl),

indolyl (1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), indazole (1-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl (1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (1-benzoxazolyl, 2-benzoxazolyl), benzothiazolyl (1-benzothiazolyl, 2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl, 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl), 5H-dibenz[b,f]azepine (5H-dibenz[b,f]azepin-1-yl, 5H-dibenz[b,f]azepine-2-yl, 5H-dibenz[b,f]azepine-3-yl, 5H-dibenz[b,f]azepine-4-yl, 5H-dibenz[b,f]azepine-5-yl), 10,11-dihydro-5H-dibenz[b,f]azepine (10,11-dihydro-5H-dibenz[b,f]azepine-1-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-2-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-3-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-4-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-5-yl).

The invention also relates to partly or fully saturated analogues of the ring systems mentioned above

The term "C_{1-4} alkyl-substituted aryl" refers to aryl substituted by at least one alkyl group having 1 to 4 carbon atoms, the terms aryl and alkyl as defined above.

The term "C_{1-4} alkyl-substituted heteroaryl" refers to heteroaryl substituted by at least one alkyl group having 1 to 4 carbon atoms, the terms heteroaryl and alkyl as defined above

The terms "C_{1-4} monoalkylamino" and "C_{1-4} dialkylamino" refer to an amino group having one or both of its hydrogens independently replaced by an alkyl group having 1 to 4 carbon atoms, alkyl being defined above, such as methylamino, dimethylamino, N-ethyl-N-methylamino, ethylamino, diethylamino, propylamino, dipropylamino, N-(n-butyl)-N-methylamino, n-butylamino, di(n-butyl)amino, sec-butylammino, t-butylamino, and the like.

The terms "acyl" or "carboxy" refer to a monovalent substituent comprising a C_{1-6}-alkyl group linked through a carbonyl group; such as e.g. acetyl, propionyl, butyryl, isobutyryl, pivaloyl, valeryl, and the like.

The term "acylamino" refers to the group $C_{1-n}C(=O)NH$-

The term "carboxamido" refers to the group -$C(=O)NHC_{1-n}$

The term "trifluoroalkoxy" refers to an C_{1-n} alkoxy group as defined above having three of its hydrogen atoms bonded to one or more of the carbon atoms replaced by fluor atoms, such as $(CF_3)O$-, $(CF_3)CH_2O$-.

The term "alkoxycarbonyl" refers to the group -C(=O)(R) where R is an C_{1-6} alkoxy group as defined above. The term "C_{1-6}-alkoxycarbonyl" as used herein refers to a monovalent substituent comprising a C_{1-6}-alkoxy group linked through a carbonyl group; such as e.g. methoxycarbonyl, carbethoxy, propoxycarbonyl, isopropoxycarbonyl, n-butoxycarbonyl, sec-butoxycarbonyl, tert-butoxycarbonyl, 3-methylbutoxycarbonyl, n-hexoxycarbonyl and the like.

The term "a C_{1-10} alkyl chain comprising at least one N, O or S atom in the chain" refers to a chain having 1 to 10 chain members, wherein at least one of the chain members is a N, S, or O atom, and the rest of the chain members are carbon. The chain may be a straight chain or a branched chain, and it may contain one or more double or triple bonds. The chain may be substituted.

The term "a C_{3-8}cycloalkyl ring comprising at least one N, O or S atom in the ring" refers to a ring having 3 to 8 ring members , wherein at least one of the ring members is a N, S, or O atom, and the rest of the ring members are carbon. The ring may be saturated or unsaturated, i.e. contain one or more double or triple bonds. The ring may be substituted.

The term "leaving group" includes, but is not limited to, halogen, sulfonate or an acyl group. Suitable leaving groups will be known to a person skilled in the art.

"Functional groups and derivatives " means groups like hydroxy, mercapto, oxo, amides, nitriles, carbonitriles, anhydrides, carboxylic acid, sulphonic acids, phosphonates, phosphoric acids, and esters of the before mentioned acids.

"Coupling agent" means an agent suitable for formation of acid derivatives from acids or activated acids and amines, phenols, alcohols, or acids including, but not limited to hydroxybenzotriazole (HOBt) and derivatives thereof and carbodiimides like dicyclohexylcarbodiimide and ethyldimethylaminopropyl carbodiimide (DCC, EDAC). Suitable coupling agents will

be known to the skilled person. Activated acids includes acid chlorides, acid anhydrides, esters, and similar derivatives.

The term "Protecting group" is intended to include any group which protect the amino group when the carboxylic group in the compound of formula II is subjected to functional derivatization, and which is easy to remove afterwards by cleavage. Such protecting groups are described in "Protective groups in organic chemistry", 2. Ed, Greene,T.W.; Wuts,P.G.M., John Wiley&Sons,Inc. 1991; and "The Peptides, Analysis, Synthesis, Biology", vol 3 "Protection of Functional Groups in Peptide synthesis", Gross,E.; Meienhofer, J.; Academic Press. A suitable protecting group is a group of formula -C(=O)-C_{1-12}alkyl, -C(=O)-O-C_{1-12}alkyl, -C(=O)-C_{1-12}alkenyl or -C(=O)-O-C_{1-12}alkenyl, optionally substituted with one or more halogen, C_{1-6}alkyl, hetaryl, aryl or fused-ring aromatic system, e.g. Troc, Boc, and Fmoc.

The term "agent capable of forming an amide or ester or mixed carbonic anhydride or anhydride or acidhalide" is intended to include such agents which activates the compound by forming a functional derivative which may be used in acylation. Such agents are described in "The Peptides, Analysis, Synthesis, Biology", vol 1 "Major Methods of Bond Formation", Gross,E.; Meienhofer, J.; Academic Press, 1981. Suitable agents are selected from benzotriazole, isobutyl chlorocarbonate, DHOBt, HOBt, HOSu, and HOAt.

The term "de-protection" is intended to include acidic, basic, oxidative or reductive cleavage as described in "The Peptides, Analysis, Synthesis, Biology", vol 1 "Major Methods of Bond Formation", Gross,E.; Meienhofer, J.; Academic Press, 1981. When, for instance, the protection group is Troc then reductive cleavage is carried out with Zn and acetic acid.

"Halogen" refers to fluorine, chlorine, bromine, and iodine. "Halo" refers to fluoro, chloro, bromo and iodo.

"Halo-alkyl" means the group -R-halo in which R is alkyl, and both alkyl and halo are as defined herein. The alkyl group may bear one, two or three halogen substituents; examples include, but are not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, chloroethyl, dichloroethyl, bromoethyl, iodoethyl, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstances may or may not occur, and that the description includes instances where said event or circumstance occur and instances in which is does not. For example, "aryl ... optionally substituted" means that the aryl may or may not be substituted and that the description includes both unsubstituted aryls and aryls wherein there is substitution

Certain of the above defined terms may occur more than once in the above formula I, and upon such occurrence each term shall be defined independently of the other.

The compounds according to the invention may be prepared by the following methods.

Method 1.

The bivalent SP inhibitor (I) is prepared by reacting A-B-X, in which X is a functional group capable of reacting with structures D-Y, in which Y is a functional group, by means of normal coupling reactions using coupling reagents known by the person skilled in the art under formation of structures I

Method 2.

Compound I can be prepared by reaction between A-B-Z , in which Z is a leaving group and D-W in which W is a nucleofile .

Examples of leaving groups are halogens , sulfonates , phosphonates,

Examples of nucleofiles are hydroxy , amino , N-substituted amino, and carbanions.

Method 3.

Compound I can be prepared by reaction between D-B-X , in which X is a functional group capable of reacting with structures A-Y, in which Y is a functional group, by means of normal coupling reactions using the coupling reagents known by the person skilled in the art under formation of structures I

Method 4.

Compound I can be prepared by reaction between D-B-Z , in which Z is a leaving group, and A-W, in which W is a nucleofile .

Examples of leaving groups are halogens , sulfonates , phosphonates,

Examples of nucleofiles are hydroxy , amino , N-substituted amino, and carbanions.

Method 5.

The linker B can be reacted with structures A and D connected to a solid phase surface using methods well known in the art.

This approach is especially valuable when the structures A and D are identical, the method here resulting in an marked increase in the yield due to the optimal distance between the solid phase coupled reagents and the linker size.

Method 6.

Compound I can be prepared by a sequence of reactions through which A or C firstly are reacted with the activated linker moiety forming A-B, respectively D-B moieties and subsequently the formed product is reacted with D, respectively A moiety.
The actual bond formation taking place through reaction on functional groups or derivatives or leaving groups /nucleofiles as described under methods 1-4.

The reaction can be carried out on a solid phase support using the procedures known by the person skilled in the art.

The compounds of the present invention may have one or more asymmetric centres and it is intended that stereoisomers (optical isomers), as separated, pure or partially purified stereoisomers or racemic mixtures thereof are included in the scope of the invention.

Within the present invention, the compounds of formula I may be prepared in the form of pharmaceutically acceptable salts, especially acid-addition salts, including salts of organic acids and mineral acids. Examples of such salts include salts of organic acids such as formic acid, fumaric acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, succinic acid, malic acid, tartaric acid, citric acid, benzoic acid, salicylic acid and the like. Suitable inorganic acid-addition salts include salts of hydrochloric, hydrobromic, sulphuric and phosphoric acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in Journal of Pharmaceutical Science, 66, 2 (1977) which are known to the skilled artisan.

Also intended as pharmaceutically acceptable acid addition salts are the hydrates which the present compounds are able to form.

The acid addition salts may be obtained as the direct products of compound synthesis. In the alternative, the free base may be dissolved in a suitable solvent containing the appropriate acid, and the salt isolated by evaporating the solvent or otherwise separating the salt and solvent.

The compounds of this invention may form solvates with standard low molecular weight solvents using methods known to the skilled artisan.

The bivalent SP inhibitors of the invention are useful for the preparation of a pharmaceutical composition for the treatment of or prophylaxis of thrombotic or coagulopathic related diseases or disorders including vascular diseases and inflammatory responses. Such diseases and responses include, but are not limited to deep venous thrombosis, arterial thrombosis, post surgical thrombosis, coronary artery bypass graft (CABG), percutaneous transdermal coronary angioplastry (PTCA), stroke, tumour metastasis, inflammation, septic chock, hypotension, ARDS, pulmonary embolism, disseminated intravascular coagulation (DIC), vascular restenosis, platelet deposition, myocardial infarction, angiogenesis, or the prophylactic treatment of mammals with atherosclerotic vessels at risk for thrombosis.

The bivalent SP inhibitors may be administered in pharmaceutically acceptable acid addition salt form or, where appropriate, as a alkali metal or alkaline earth metal or lower alkylammonium salt. Such salt forms are believed to exhibit approximately the same order of activity as the free base forms.

Apart from the pharmaceutical use of the compounds, they may be useful in vitro tools for investigating the inhibition of FVIIa, FXa or FVIIa/TF/FXa activity.

Pharmaceutical compositions

In another aspect, the present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

Optionally, the pharmaceutical composition of the invention may comprise a bivalent SP inhibitor in combination with one or more other compounds exhibiting anticoagulant activity, e.g., platelet aggregation inhibitor.

The compounds of the invention may be formulated into pharmaceutical composition comprising the compounds and a pharmaceutically acceptable carrier or diluent. Such carriers include water, physiological saline, ethanol, polyols, e.g., glycerol or propylene glycol, or vegetable oils. As used herein, "pharmaceutically acceptable carriers" also encompasses any and all solvents, dispersion media, coatings, antifungal agents, preservatives, isotonic agents and the like. Except insofar as any conventional medium is incompatible with the active ingredient and its intended use, its use in the compositions of the present invention is contemplated.

The compositions may be prepared by conventional techniques and appear in conventional forms, for example, capsules, tablets, solutions or suspensions. The pharmaceutical carrier employed may be a conventional solid or liquid carrier. Examples of solid carriers are lactose, terra alba, sucrose, talc, gelatine, agar, pectin, acacia, magnesium stearate and stearic acid. Examples of liquid carriers are syrup, peanut oil, olive oil and water. Similarly, the carrier or diluent may include any time delay material known to the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax. The formulations may also include wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavouring agents. The formulations of the invention may be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

The pharmaceutical compositions can be sterilised and mixed, if desired, with auxiliary agents, emulsifiers, salt for influencing osmotic pressure, buffers and/or colouring substances and the like, which do not deleteriously react with the active compounds.

The route of administration may be any route, which effectively transports the active compound to the appropriate or desired site of action, such as oral or parenteral, e.g., rectal, transdermal, subcutaneous, intranasal, intramuscular, topical, intravenous, intraurethral, ophthalmic solution or an ointment, the oral route being preferred.

If a solid carrier for oral administration is used, the preparation can be tabletted, placed in a hard gelatine capsule in powder or pellet form or it can be in the form of a troche or lozenge. The amount of solid carrier may vary widely but will usually be from about 25 mg to about 1 g. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatine capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

For nasal administration, the preparation may contain a compound of formula (I) dissolved or suspended in a liquid carrier, in particular an aqueous carrier, for aerosol application. The carrier may contain additives such as solubilizing agents, e.g. propylene glycol, surfactants, absorption enhancers such as lecithin (phosphatidylcholine) or cyclodextrin, or preservatives such as parabenes.

For parenteral application, particularly suitable are injectable solutions or suspensions, preferably aqueous solutions with the active compound dissolved in polyhydroxylated castor oil.

Tablets, dragees, or capsules having talc and/or a carbohydrate carrier or binder or the like are particularly suitable for oral application. Preferable carriers for tablets, dragees, or capsules include lactose, corn starch, and/or potato starch. A syrup or elixir can be used in cases where a sweetened vehicle can be employed.

A typical tablet, which may be prepared by conventional tabletting techniques, contains

Core:

Active compound (as free compound or salt thereof)	10 mg
Colloidal silicon dioxide (Areosil®)	1.5 mg
Cellulose, microcryst. (Avicel®)	70 mg
Modified cellulose gum (Ac-Di-Sol®)	7.5 mg
Magnesium stearate	

Coating:

HPMC	approx. 9 mg
*Mywacett® 9-40 T	approx. 0.9 mg

*Acylated monoglyceride used as plasticizer for film coating.

The compounds of the invention may be administered to a mammal, especially a human in need of such treatment, prevention, elimination, alleviation or amelioration of various thrombolytic or coagulophatic diseases or disorders as mentioned above. Such mammals also include animals, both domestic animals, e.g. household pets, and non-domestic animals such as wildlife.

Usually, dosage forms suitable for oral, nasal, pulmonal or transdermal administration comprise from about 0.001 mg to about 100 mg, preferably from about 0.01 mg to about 50 mg of the compounds of formula I admixed with a pharmaceutically acceptable carrier or diluent.

The compounds may be administered concurrently, simultaneously, or together with a pharmaceutically acceptable carrier or diluent, whether by oral, rectal, or parenteral (including subcutaneous) route. The compounds are often, and preferably, in the form of an alkali metal or earth alkali metal salt thereof.

Suitable dosage ranges varies as indicated above depending upon the exact mode of administration, form in which administered, the indication towards which the administration is directed, the subject involved and the body weight of the subject involved, and the preference and experience of the physician or veterinarian in charge.

The compounds of the present invention have interesting pharmacological properties. For example, the compounds of this invention can be used to modulate and normalise an impaired haemostatic balance in mammals caused by deficiency or malfunction of blood clotting factors or their inhibitors. The FVIIa and in particular the FVIIa/TF activity plays an important role in the control of the coagulation cascade, and modulators of this key regulatory activity such as the present invention can be used in the treatment of or prophylaxis of thrombotic or coagulopathic related diseases or disorders including vascular diseases and inflammatory responses. The pharmaceutical composition of the invention may thus be useful for modulating and normalising an impaired haemostatic balance in a mammal. In particular, the pharmaceutical composition may be useful for the treatment of or prophylaxis of thrombotic or coagulopathic related diseases or disorders including vascular diseases and inflammatory responses.

More particularly, the pharmaceutical composition may be useful as an inhibitor of blood coagulation in a mammal, as an inhibitor of clotting activity in a mammal, as an inhibitor of deposition of fibrin in a mammal, as an inhibitor of platelet deposition in a mammal, in the treatment of mammals suffering from deep venous thrombosis, arterial thrombosis, post surgical thrombosis, coronary artery bypass graft (CABG), percutaneous transdermal coronary angioplastry (PTCA), stroke, tumour metastasis, inflammation, septic chock, hypotension, ARDS, pulmonary embolism, disseminated intravascular coagulation (DIC), vascular restenosis, platelet deposition, myocardial infarction, angiogenesis, or the prophylactic treatment of mammals with atherosclerotic vessels at risk for thrombosis. The compositions of the invention may also be used as an adjunct in thrombolytic therapy.

Furthermore the invention relates to a method for inhibiting the TF initiation activity in a mammal which method comprises administering an effective amount of at least one compound of the present invention, in combination with a pharmaceutical acceptable excipient and/ or carrier to the mammal in need of such a treatment.

The invention also relates to a method for inhibiting FVIIa activity by substantially reducing the ability of activated FVIIa to catalyse TF-enhanced activation of factors X and IX, the method comprising administering at least one compound of the invention, in combination with a pharmaceutical acceptable excipient and/ or carrier to a mammal in need of such a treatment.

Assays

A "FVIIa inhibitor" may be identified as a substance A which reduces the amidolytic activity by at least 50% at a concentration of substance A at 400 μM in the FVIIa amidolytic assay described by Persson et al. (Persson et al., J. Biol. Chem. 272: 19919-19924 (1997)). Preferred are substances reducing the amidolytic activity by at least 50% at a concentration of substance A at 300 μM; more preferred are substances reducing the amidolytic activity by at least 50% at a concentration of substance A at 200 μM.

A "FXa inhibitor" may be identified as a substance D which reduces the amidolytic activity by at least 50% at a concentration of substance D at 400 μM in the FXa amidolytic assay described by Soerensen et al. (Sørensen et al., J. Biol. Chem. 272: 11863-11868 (1997)). Preferred are substances reducing the amidolytic activity by at least 50% at a concentration of

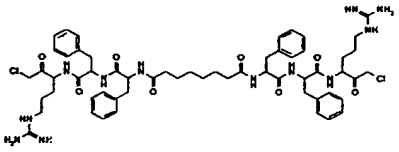
substance D at 300 μM; more preferred are substances reducing the amidolytic activity by at least 50% at a concentration of substance D at 200 μM.

The present invention is further illustrated by the following examples.

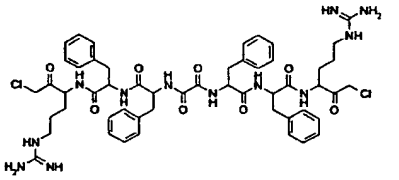
The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a number of aspects of the invention and any embodiments which are functionally equivalent are within the scope of this invention. Those skilled in the art will know, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

EXAMPLES

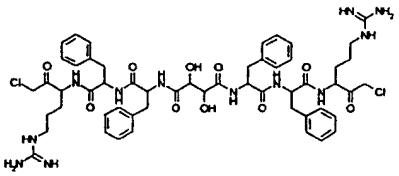
The following bivalent SP inhibitors were synthesised:



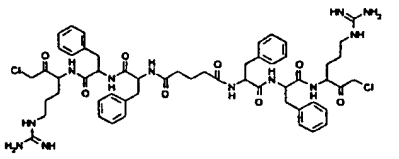
Octanedioic acid bis-({1-[1-(1-chloroacetyl-4-guanidino-butylcarbamoyl)-2-phenyl-ethylcarbamoyl]-2-phenyl-ethyl}-amide) (Example 1)



N, N'-bis-(1-{1-[1-(2-chloro-acetyl)-4-guanidino-butylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-phenylethyl)-oxalamide (Example 2)



N, N'-bis-(1-{1-[1-(2-chloro-acetyl)-4-guanidino-butylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-phenylethyl-2,3-dihydroxysuccinamide (Example 3)



Pentanedioic acid bis-[(1-{1-[1-(2-chloro-acetyl)-4-guanidino-butylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-phenyl-ethyl)-amide] (Example 4)

Example 1

Octanedioic acid bis-({1-[1-(1-chloroacetyl-4-guanidino-butylcarbamoyl)-2-phenyl-ethylcarbamoyl]-2-phenyl-ethyl}-amide) (1)

Disuccinimidyl suberate (Pierce #21555) (5.86 mg) was mixed with FFR-CMK (25 mg) in 1.5 ml of phosphate buffer pH 7.4, after addition of two drops of DMF the mixture was stirred in a closed vessel under N2 atmosphere for 1 day, subsequent evaporation yielded an yellow oil raw product (35 mg) which was purified on HPLC (reversed-phase column (Symmetry-Shild ,C_8, Waters, Part no. WAT200655)) with a constant flow of 1 ml/min. Elution was accomplished by increasing the percentage of organic phase (acetonitrile contaning 0.1 % trifluoroacetic acid (TFA)) relative to aqueous phase (0.1 % TFA in H_2O). A linear gradient from 14% to 50% organic phase over 35 min was used where the dimeric form of FFR-CMK was eluted at about 28 min .)

The fraction at rt (retention time) 30.94 min was isolated. MS (M+1) 1141 yield 12 % oil.

Example 2

N,N'-Bis-(1-{1-[1-(2-chloro-acetyl)-4-guanidino-butylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-phenylethyl)-oxalamide (2)

Disuccinimidyl oxalate (4.2 mg) and FFR-CMK (25 mg) were mixed in 1.5 ml of phosphate buffer pH 7.4, after addition of two drops of DMF the mixture was stirred in a closed vessel under N2 atmosphere for 2 days, subsequent evaporation yielded an yellow oil raw product which was purified on HPLC (reversed-phase column (SymmetryShild,C_8, Waters, Part no. WAT200655)) with a constant flow of 1 ml/min. Elution was accomplished by increasing the percentage of organic phase (acetonitrile contaning 0.1 % trifluoroacetic acid (TFA)) relative to aqueous phase (0.1 % TFA in H_2O). A linear gradient from 14% to 50% organic phase over 35 min was used where the dimeric form of FFR-CMK was eluted at about 28 min .)

The fraction at rt 31.08 min was isolated. MS (M+1) 1057, yield 8% yellow oil.

Example 3

N,N'-Bis-(1-{1-[1-(2-chloro-acetyl)-4-guanidino-butylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-phenylethyl-2,3-dihydroxysuccinamide (3)

Disuccinimidyl tartrate (Pierce # 20589) (5.16 mg) and FFR-CMK (25 mg) were mixed in 1.5 ml of phosphate buffer pH 7.4, after addition of 4 drops of DMF the mixture was stirred in a closed vessel under N2 atmosphere overnight, subsequent evaporation yielded an yellow oil raw product which was purified on HPLC (reversed-phase column (SymmetryShild,C_8,

Waters, Part no. WAT200655)) with a constant flow of 1 ml/min. Elution was accomplished by increasing the percentage of organic phase (acetonitrile contaning 0.1 % trifluoroacetic acid (TFA)) relative to aqueous phase (0.1 % TFA in H_2O). A linear gradient from 14% to 50% organic phase over 35 min was used where the dimeric form of FFR-CMK was eluted at about 28 min .)

The fraction at rt 29.20 min was isolated. MS (M+1) 1117, yield 4 % yellow oil.

Example 4

Pentanedioic acid bis-[(1-{1-[1-(2-chloro-acetyl)-4-guanidino-butylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-phenyl-ethyl)-amide] (4)

Disuccinimidyl glutarate (Pierce # 20593) (4.89 mg) and FFR-CMK (25 mg) were mixed in 1.5 ml of phosphate buffer pH 7.4, after addition of1 ml of DMF the mixture was stirred in a closed vessel under N2 atmosphere for 3 days, subsequent evaporation yielded an yellow oil raw product which was purified on HPLC (reversed-phase column (SymmetryShild,C_8, Waters, Part no. WAT200655)) with a constant flow of 1 ml/min. Elution was accomplished by increasing the percentage of organic phase (acetonitrile contaning 0.1 % trifluoroacetic acid (TFA)) relative to aqueous phase (0.1 % TFA in H_2O). A linear gradient from 14% to 50% organic phase over 35 min was used where the dimeric form of FFR-CMK was eluted at about 28 min .)

The fraction at rt 29.20 min was isolated. MS (M+1) 1099, yield 18 % yellow oil.

Example 5

Production of dim-FFR-FVIIa.

The synthesised and purified dim-FFR (**1**) was mixed with FVIIa using 45 μM and 15 μM of dim-FFR and FVIIa, respectively in 100 mM HEPES, 100 mM NaCl, 1.5 mM $CaCl_2$, pH 7.5. They were allow to react over night at 4 °C before measuring the FVIIa amidolytic activity. The FVIIa amidolytic activity was measured using 100 nM dim-FFR-FVIIa and 1 mM of the chromogenic substrate S-2288 (Chromogenix). The milliOD_{405}/min values from these measurements showed that FVIIa was completely inhibited by dim-FFR (0,0135 ± 0,019 milliOD_{405}/min and 18,86 ± 0,39 milliOD_{405}/min for 100 nM dim-FFR-FVIIa and FVIIa, respectively). The excess of dim-FFR was removed by gel filtration (Pharmacia NAP-10, code no. 17-0854-02) in order to obtain a pure dim-FFR-FVIIa form.

Example 6

Inhibition of FXa selectively on the FVIIa/TF Complex

Factor Xa inhibition by dim-FFR-FVIIa (1) was detected using a chromogenic assay. Tissue factor was purchased from America Diagnostica Inc. (# 4500) and was reconstituted into membranes (Sigma) containing 75% (w/w) PC and 25% (w/w) PS (PC/PS) and used after exhaustive dialysis (Lawson et al., Methods Enzymol. 222: 177-195). A reaction mixture containing 10 nM FXa, 20 nM dim-FFR-FVIIa and with and without 5 nM TF(PC/PS) (50 mM HEPES, 100 mM NaCl, 5 mM $CaCl_2$, 0.01 % Tween 80, pH 7.5) was incubated over night at room temperature. The chromogenic substrate S-2288 (Chromogenix) was then added to a final concentration of 1 mM and the results were analysed by monitoring the change at 405 nm. These data is shown in Figure 3 as milliOD_{405}/min. It is clearly demonstrated in this example that dim-FFR-FVIIa is selectively inhibiting FXa only in presence of TF(PC/PS). There is only a small inhibition of FXa by dim-FFR-FVIIa in absence of TF(PC/PS). To determine that FVIIa and FXa becomes covalently joined through the dim-FFR protease inhibitor linker, a western blot analysis was performed. Inhibited sample as shown in Figure 3 was electrophoresed on 4-20 % SDS-polyacrylamide gel (Novex, cat. No. EC6025) and transferred onto nitro-cellulose membrane (Millipore). A dimeric form of protein was detected using a anti-FVIIa IgG (F7A2B17). The result presented in Figure 3 (as inset) show that a dimeric form of FVIIa and FXa is formed with a molecular weight of about 100 kDa (lane S), The calculated molecular with of a dimeric form of FVIIa and FXa is 105 kDa. A reference FVIIa is also included in the western blot (lane C) showing a molecular weight of about 50 kDa, agreeable with the molecular with of FVIIa. One can also conclude form this experiments that a linker of 11.5 Å using for this dim-FFR compound is long enough to achieve simultaneous inhibition of FVIIa and FXa in the TF complex.

Example 7

Specific Inhibition of FXa on the FVIIa/TF Complex

The specificity of the inhibition of FXa on the dim-FFR-FVIIa/TF complex was evaluated by comparing with to other serine proteases involved in the coagulation cascade, thrombin and protein C. Specific factor Xa inhibition by dim-FFR-FVIIa (1) was measured using a chromogenic assay. Tissue factor was purchased from America Diagnostica Inc. (# 4500) and was reconstituted into membranes (Sigma) containing 75% (w/w) PC and 25% (w/w) PS (PC/PS) and used after exhaustive dialysis (Lawson et al., Methods Enzymol. 222: 177-195). A reaction mixture containing 2 nM different proteases (indicated in Figure 3), 8 nM dim-

FFR-FVIIa and with and without 4 nM TF(PC/PS) (50 mM HEPES, 100 mM NaCl, 5 mM $CaCl_2$, 0,01 % Tween 80, pH 7.5) was incubated for 3 hours at room temperature. The chromogenic substrate S-2288 (Chromogenix) was then added to a final concentration of 1 mM and the results were analyzed by monitoring the change at 405 nm. The amidolytic date for each protease is shown in Figure 4 as the milliOD_{405}/min value relative to the protease without dim-FFR-FVIIa.. Among these serine proteases, only FXa is selectively inhibited by dim-FFR-FVIIa in the presence of TF(PC/PS). This specific inhibition of FXa is not due to the inhibition profile of FFR *per se*, it is due to the selective binding trough macromolecule interaction of FXa to the FVIIa/TF complex. FXa, thrombin and protein C is all inhibited to the same extent by the free form of FFR or dim-FFR.

Example 8

Time Dependency of Inhibition of FXa on the FVIIa/TF Complex

Factor Xa inhibition by dim-FFR-FVIIa (**1**) was established using a chromogenic assay. Tissue factor was purchased from America Diagnostica Inc. (# 4500) and was reconstituted into membranes (Sigma) containing 75% (w/w) PC and 25% (w/w) PS (PC/PS) and exhaustive dialysis (Lawson et al., Methods Enzymol. 222: 177-195). A reaction mixture containing 2 nM FXa, 8 nM dim-FFR-FVIIa, 8 nM dim-FFR and with and without 4 nM TF(PC/PS) (50 mM HEPES, 100 mM NaCl, 5 mM $CaCl_2$, 0,01 % Tween 80, pH 7.5) was incubated for various times at room temperature. The chromogenic substrate S-2765 (Chromogenix) was added to a final concentration of 0,5 mM and analyzed by monitoring the change at 405 nm. Figure 5 shows the rate of inhibition as decrease in milliOD_{405}/min. The sample lacking TF(PC/PS) show no detectable inhibition of FXa while in the presence of TF(PC/PS) the half-time of inactivation is estimated to about 75 minutes under these experimental conditions. When using 8 nM free form of dim-FFR, there was no detectable inhibition (Figure 5), showing the importance of position the dim-FFR on the TF complex.

Example 9

Concentration Dependency of Inhibition of FXa on the FVIIa/TF Complex

Factor Xa inhibition by dim-FFR-FVIIa (**1**) was established using a chromogenic assay. Tissue factor was purchased from America Diagnostica Inc. (# 4500) and was reconstituted into membranes (Sigma) containing 75% (w/w) PC and 25% (w/w) PS (PC/PS) and exhaustive dialysis (Lawson et al., Methods Enzymol. 222: 177-195). A reaction mixture containing 2 nM FXa and various concentration of dim-FFR-FVIIa and dim-FFR with and without

TF(PC/PS) (50 mM HEPES, 100 mM NaCl, 5 mM $CaCl_2$, 0,01 % Tween 80, pH 7.5) was incubated for 3 hours at room temperature. The TF(PC/PS) concentration was half the concentration used for dim-FFR-FVIIa. The chromogenic substrate S-2765 (Chromogenix) was added to a final concentration of 0,5 mM and analyzed by monitoring the change at 405 nm. Figure 6 shows the FXa inhibition as a function of increasing dim-FFR-FVIIa and free dim-FFR with or without TF(PC/PS) as the decrease in milliOD_{405}/min. The IC_{50} value without TF(PC/PS) under these experimental condition is about 80 nM, both for dim-FFR-FVIIa and the free form of dim-FFR, argue that there is the same inhibition mechanism regardless if dim-FFR is situated on FVIIa or is free in solution. When adding TF(PC/PS) to the sample, the IC_{50}-value drops 20-fold down to about 4 nM, again showing the enhancement of FXa inhibition when FVIIa and FXa becomes oriented on TF.

Example 10

Identification of Optimal Covalent-Linked Bivalent SP Inhibitor for the FVIIa/TF/FXa Complex.

The complex between FVIIa/TF/FXa on the membrane surface that are transiently formed initially when TF becomes exposed holds two SP active sites, one from FVIIa and one from FXa. The precise orientation of the FVIIa and FXa protease domains in the FVIIa/TF/FXa complex is not known. We only know from the FVIIa/TF complex structure (Banner et al., Nature 380: 41-46 (1996)) how the protease domain of FVIIa is oriented on TF, there is no available structural data on the FXa binding to the FVIIa/TF complex. The fact that FVIIa activates FX to FXa by proteolytically cleavage of a peptide bond in the protease domain of FXa, argues that the protease domains of FVIIa and FXa must come in physical contact with each other when bound to TF. One can only speculate about the exact distance between the active site of FVIIa and the active site of FXa when these two coagulation factors are present on TF. The exact alignment of FXa on the FVIIa/TF complex and the rotation of the FVIIa and FXa protease domains relative each other will determine this active site distance. To determine this distance, we used a series of dim-FFRs with different length of the linker. The length of the linkers is defined as the distance between the primary α-amine in the FFR moiety or the distance between the γO atoms of the serine residue (no. 195 according to α-chymotrypsin numbering) involved in the catalytic triad in FVIIa and FXa. The distance between the primary α-amine in the FFR moiety and the γO atom of the serine residue 195 is 9.0 Å, determined from the FVIIa/TF complex structure (Banner et al., Nature 380: 41-46 (1996)). Factor Xa inhibition by various dim-FFR-FVIIa was established using a chromogenic

assay. Tissue factor was purchased from America Diagnostica Inc. (# 4500) and was reconstituted into membranes (Sigma) containing 75% (w/w) PC and 25% (w/w) PS (PC/PS) and exhaustive dialysis (Lawson et al., Methods Enzymol. 222: 177-195). A reaction mixture containing 2 nM FXa and 8nM of the different dim-FFR-FVIIa with and without 8 nM TF(PC/PS) (50 mM HEPES, 100 mM NaCl, 5 mM $CaCl_2$, 0,01 % Tween 80, pH 7.5) was incubated for 6 hours at room temperature. The chromogenic substrate S-2765 (Chromogenix) was added to a final concentration of 0,5 mM and analyzed by monitoring the change at 405 nm. Figure 7 shows that the optimal linker for FXa inhibition on the FVIIa/TF complex is extremely dependent on the length. FXa inhibition increase abruptly between 6.2 Å and 7.4 Å (defined as the distance between the primary α-amine in the FFR moiety) or 24.2 Å and 25.4 Å (defined as the distance between the γO atoms of the serine residue 195 in the active site of FVIIa and FXa). This steep transition probably result from the precise binding of FXa to the FVIIa/TF complex, allowing only a very defined length of the linker between two SP inhibitors in this particular case. This is remarkable considering the binding of this bivalent SP inhibitor is directed toward two different proteases. In the absence of TF (Figure 7), the linker-length inhibition dependency is no longer observed, and no inhibition of FXa is detected. This again showing the localization effect TF has on these two proteases, bringing them together allowing effective active site inhibition simultaneously of FVIIa and FXa using low-molecular compounds. Figure 7 also shows that by increasing the linker length to 11.2 Å (defined as the distance between the primary α-amine in the FFR moiety) or 29.2 Å (defined as the distance between the γO atoms of the serine residue 195 in the active site of FVIIa and FXa) there is only a small increase in inhibition compared to the 7.4 Å linker (defined as the distance between the primary α-amine in the FFR moiety) or 24.2 Å (defined as the distance between the γO atoms of the serine residue 195 in the active site of FVIIa and FXa). Hence, our bivalent SP inhibitor strategy should reveal compounds that are more selective and more potent than monomeric SP inhibitors if the linker length used is longer that 6 Å (defined as the distance between the primary α-amine in the FFR moiety) or 24 Å (defined as the distance between the γO atoms of the serine residue 195 in the active site of FVIIa and FXa) and shorter that about 40 Å (defined as the distance between the primary α-amine in the FFR moiety) or 58 Å (defined as the distance between the γO atoms of the serine residue 195 in the active site of FVIIa and FXa).

CLAIMS

1. A compound having the formula A-(LM)-D that binds via FVIIa and FXa to a complex comprising TF, FVIIa and FXa, wherein A is a moiety that binds FVIIa; D is a moiety that binds FXa; and LM is a linker moiety, and wherein the affinity of binding of said compound to said complex is at least about 10-fold higher than the affinity of binding of said compound to either free FVIIa or free FXa.

2. A compound according to claim 1, wherein A binds to the active site of FVIIa.

3. A compound according to claim 1, wherein D binds to the active site of FXa.

4. A compound according to claim 1, wherein A binds to the active site of FVIIa and D binds to the active site of FXa.

5. A compound according to claim 1, which co-localises the FVIIa and FXa on TF in such a way that the distance measured between the γ-oxygen atoms of the serine residue 195 in the active site of FVIIa and FXa, respectively, is at least about 24 angstroms.

6. A compound according to claim 5, which co-localises the FVIIa and FXa on TF in such a way that the distance measured between the γ-oxygen atoms of the serine residue 195 in the active site of FVIIa and FXa, respectively, is at least about 24 angstroms and at most about 58 angstroms.

7. A compound according to claim 1, wherein LM is a peptide or LM is selected among the following structures:
C_{1-18} alkyl, a 1 to 18 -membered straight or branched chain comprising carbon and at least one N, O or S atom in the chain, C_{3-8}cycloalkyl, a 3 to 8 -membered cyclic ring comprising carbon and at least one N, O or S atom in the ring, aryl, C_{1-4}alkyl-substituted aryl, heteroaryl, C_{1-4} alkyl-substituted heteroaryl;
the structures optionally substituted with one or more of the following groups: hydroxy, oxo, amino, C_{1-4} monoalkylamino, C_{1-4} dialkylamino, acylamino, sulfonyl, carboxy, carboxamido , halogeno, C_{1-6} alkoxy, C_{1-6} alkylthio, trifluoroalkoxy, alkoxycarbonyl, haloalkyl.

8. A compound according to claim 1, which is dim-FFR.

9. A compound according to claim 1, wherein the reduction of coagulation activity is a reduction of proteolytic activity of either or both of FVIIa and FXa.

10. A compound according to claim 9, that reduces proteolytic activity of FVIIa in FVIIa/TF/FXa complex

11. A compound according to claim 9, that reduces proteolytic activity of FXa in FVIIa/TF/FXa complex

12. A method for reducing TF-mediated coagulation activity, said method comprising contacting a FVIIa/TF/FXa complex with a compound having the formula A-(LM)-D, wherein A is a moiety that binds FVIIa; D is a moiety that binds FXa; and LM is a linker moiety, and wherein the affinity of binding of said compound to said complex is at least about 10-fold higher than the affinity of binding of said compound to either free FVIIa or free FXa.

13. A method according to claim 12, wherein the reduction of coagulation activity is a reduction of proteolytic activity of either or both of FVIIa and FXa.

14. A method according to claim 13, wherein the reduction of coagulation activity is a reduction of proteolytic activity of FVIIa.

15. A method according to claim 13, wherein the reduction of coagulation activity is a reduction of proteolytic activity of FXa.

16. A method according to claim 12, wherein the FVIIa and FXa are co-localised on TF in such a way that the distance measured between the γ-oxygen atoms of the serine residue 195 in the active site of FVIIa and FXa, respectively, is at least about 24 angstroms.

17. A compound according to claim 12, wherein the FVIIa and FXa are co-localised on TF in such a way that the distance measured between the γ-oxygen atoms of the serine residue 195 in the active site of FVIIa and FXa, respectively, is at least about 24 angstroms and at

most about 58 angstroms.

18. A method according to claim 12, wherein LM is a peptide or LM is selected among the following structures:

C_{1-18} alkyl, a 1 to 18 -membered straight or branched chain comprising carbon and at least one N, O or S atom in the chain, C_{3-8}cycloalkyl, a 3 to 8 -membered cyclic ring comprising carbon and at least one N, O or S atom in the ring, aryl, C_{1-4}alkyl-substituted aryl, heteroaryl, C_{1-4} alkyl-substituted heteroaryl;

the structures optionally substituted with one or more of the following groups: hydroxy, oxo, amino, C_{1-4} monoalkylamino, C_{1-4} dialkylamino, acylamino, sulfonyl, carboxy, carboxamido , halogeno, C_{1-6} alkoxy, C_{1-6} alkylthio, trifluoroalkoxy, alkoxycarbonyl, haloalkyl.

19. A pharmaceutical composition for inhibiting TF-mediated coagulation activity, comprising an amount of the compound of claims 1-11 and a pharmaceutically acceptable carrier or excipient.

20. A compositions according to claim 19, wherein the composition further contains a platelet aggregation inhibitor.

21. Use of a compound as defined in claims 1-11 for the manufacture of a medicament for preventing or treating FVIIa/TF related diseases or disorders

22. Use according to claim 21, wherein the FVIIa/TF related diseases or disorders are deep venous thrombosis, arterial thrombosis, post surgical thrombosis, coronary artery bypass graft (CABG), percutaneous transdermal coronary angioplastry (PTCA), stroke, tumour metastasis, angiogenesis, thrombolysis, arteriosclerosis and restenosis following angioplastry, acute and chronic indications such as inflammation, septic chock, septicemia, hypotension, adult respiratory distress syndrome (ARDS), disseminated intravascular coagulopathy (DIC), pulmonary embolism, platelet deposition, myocardial infarction, or the prophylactic treatment of mammals with atherosclerotic vessels at risk for thrombosis

23. A method for prevention or treatment of FVIIa/TF related diseases or disorders in a mammal, which method comprises administering an effective amount of at least one

compound as defined in claim 1-11 to the mammal.

24. A method according to claim 23, wherein the FVIIa/TF related diseases or disorders are deep venous thrombosis, arterial thrombosis, post surgical thrombosis, coronary artery bypass graft (CABG), percutaneous transdermal coronary angioplastry (PTCA), stroke, tumour metastasis, angiogenesis, thrombolysis, arteriosclerosis and restenosis following angioplastry, acute and chronic indications such as inflammation, septic chock, septicemia, hypotension, adult respiratory distress syndrome (ARDS), disseminated intravascular coagulopathy (DIC), pulmonary embolism, platelet deposition, myocardial infarction, or the prophylactic treatment of mammals with atherosclerotic vessels at risk for thrombosis, which method comprises administering a therapeutically effective amount of at least one compound as defined in claims 1-11 in combination with a pharmaceutical acceptable excipient and/ or carrier, to the mammal in need of such a treatment.

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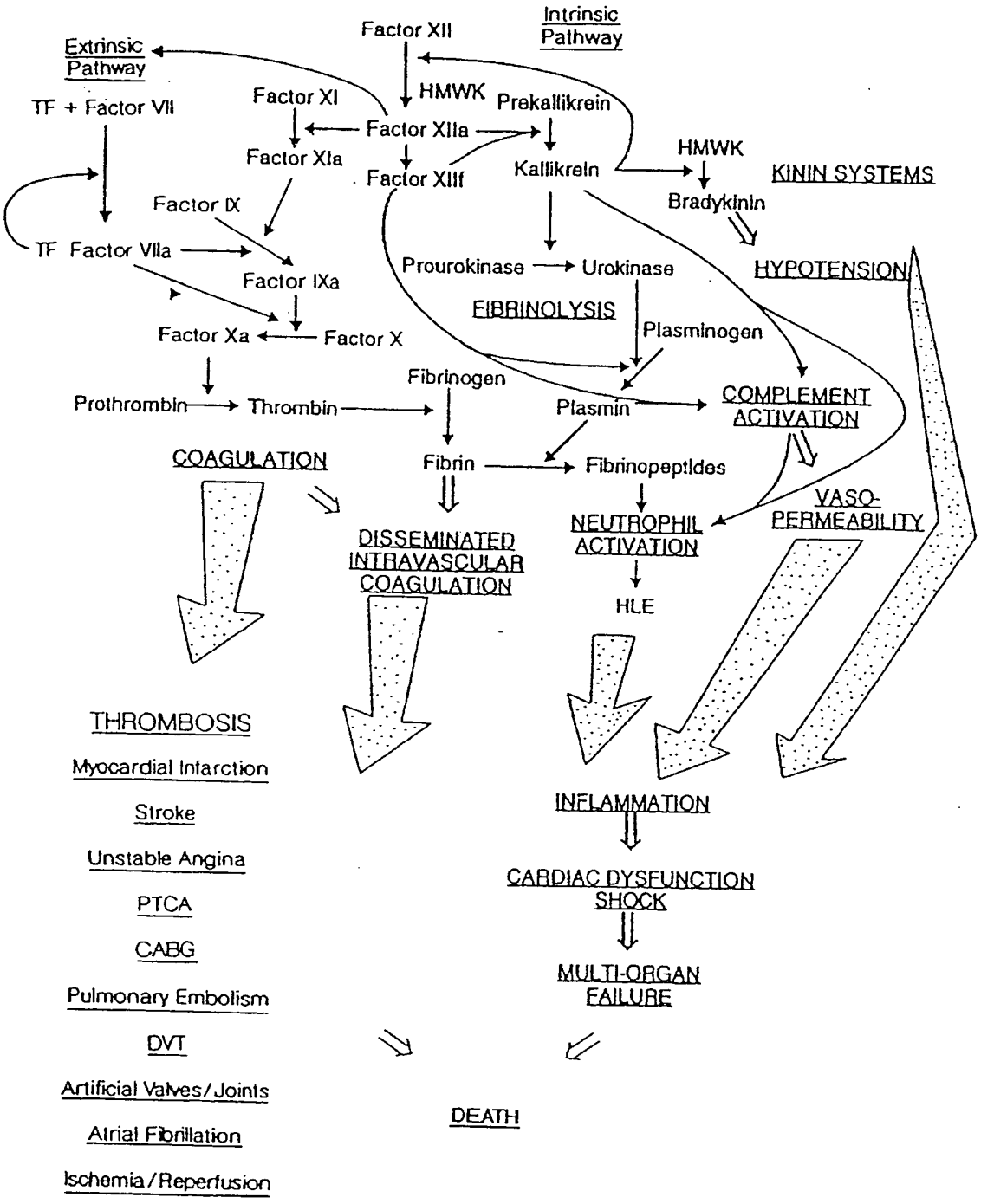
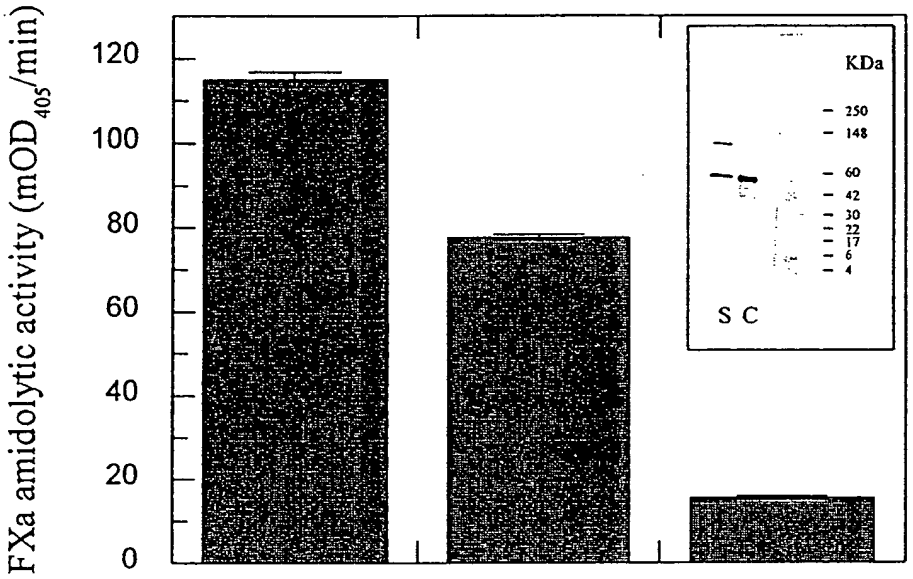

Extrinsic Pathway
Factor XII
Intrinsic Pathway
TF + Factor VII
Factor XI
HMWK
Prekallikrein
Factor XIIa
Factor XIa
Factor XIIf
Kallikrein
HMWK
KININ SYSTEMS
Bradykinin
Factor IX
TF Factor VIIa
Factor IXa
Prourokinase
Urokinase
HYPOTENSION
FIBRINOLYSIS
Plasminogen
Factor Xa
Factor X
Fibrinogen
Prothrombin
Thrombin
Plasmin
COMPLEMENT ACTIVATION
COAGULATION
Fibrin
Fibrinopeptides
VASO-PERMEABILITY
DISSEMINATED INTRAVASCULAR COAGULATION
NEUTROPHIL ACTIVATION
HLE
THROMBOSIS
Myocardial Infarction
Stroke
Unstable Angina
PTCA
CABG
Pulmonary Embolism
DVT
Artificial Valves/Joints
Atrial Fibrillation
Ischemia/Reperfusion
INFLAMMATION
CARDIAC DYSFUNCTION SHOCK
MULTI-ORGAN FAILURE
DEATH

Fig. 1

Fig. 2



10 nM FXa	+	+	+
20 nM dFFR-FVIIa		+	+
5 nM TF (PS/PC)			+

Fig. 3

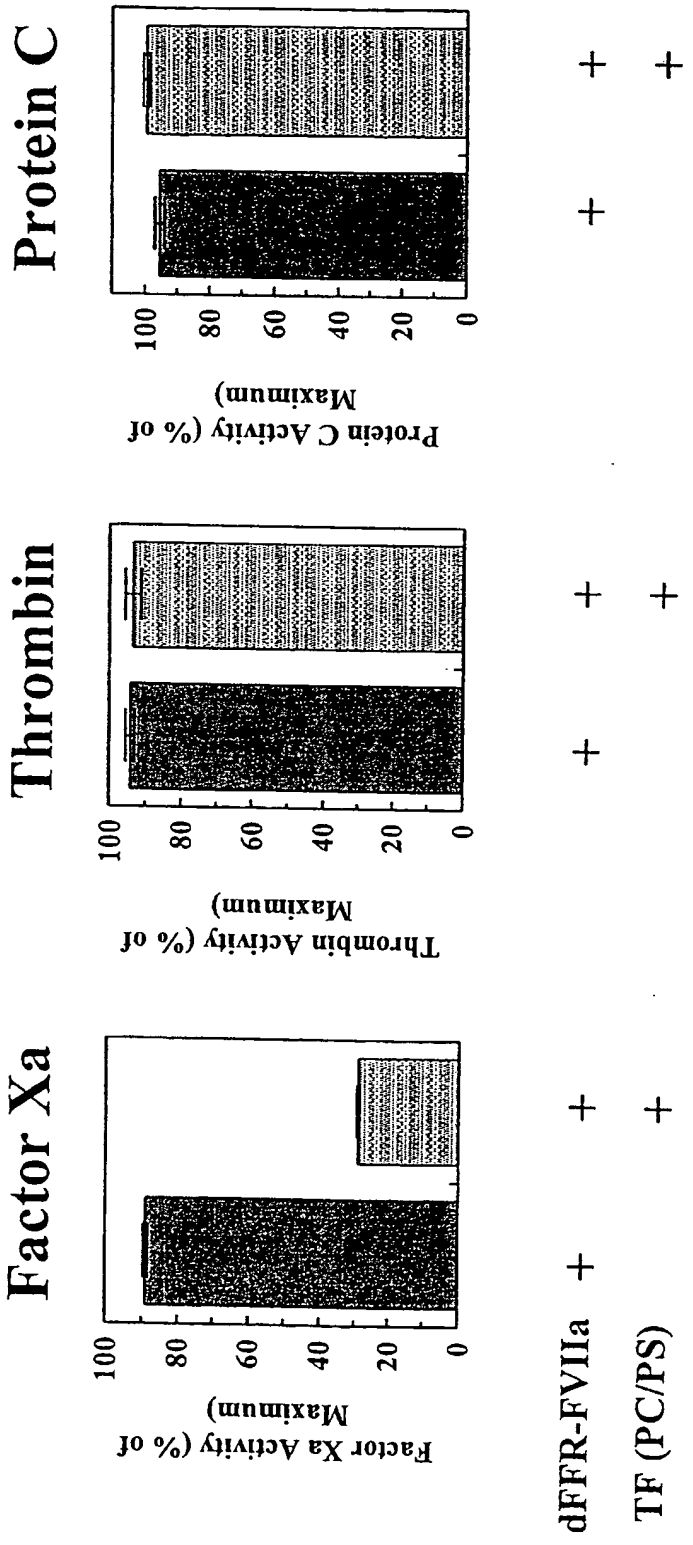

Factor Xa
Factor Xa Activity (% of Maximum)
100
80
60
40
20
0
Thrombin
Thrombin Activity (% of Maximum)
100
80
60
40
20
0
Protein C
Protein C Activity (% of Maximum)
100
80
60
40
20
0
dFFR-FVIIa + + +
TF (PC/PS) + + +

Fig. 4

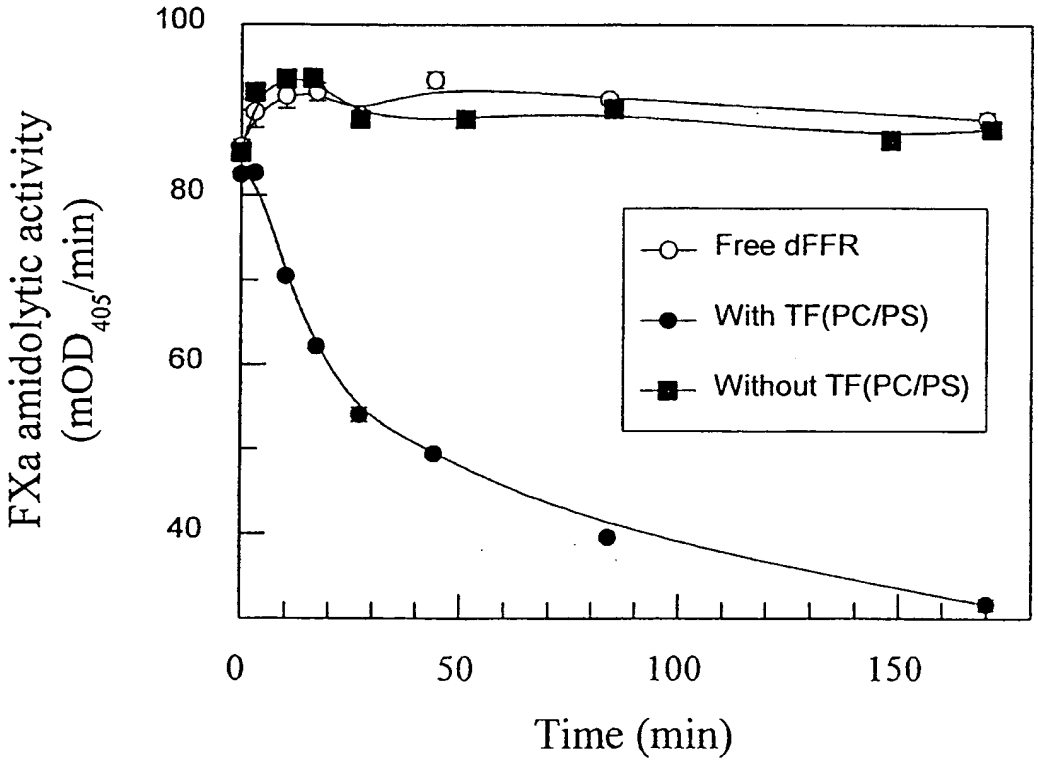

100
80
60
40
0
50
100
150
Time (min)
FXa amidolytic activity
(mOD405/min)
Free dFFR
With TF(PC/PS)
Without TF(PC/PS)

Fig. 5

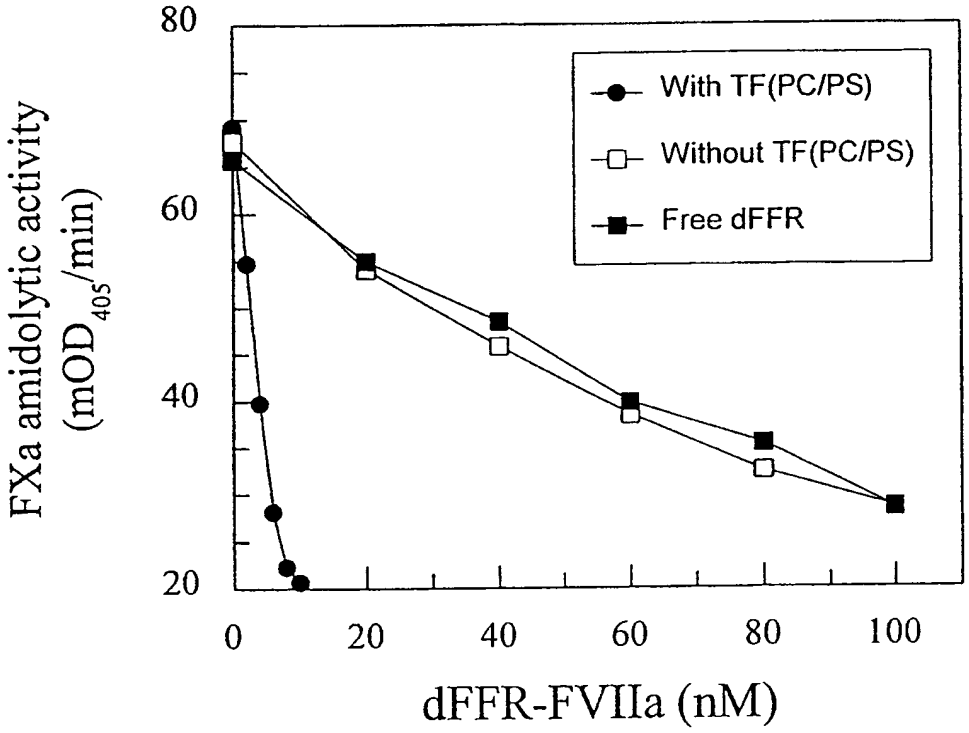

80
60
40
20
0
20
40
60
80
100
FXa amidolytic activity
(mOD405/min)
dFFR-FVIIa (nM)
With TF(PC/PS)
Without TF(PC/PS)
Free dFFR

Fig. 6

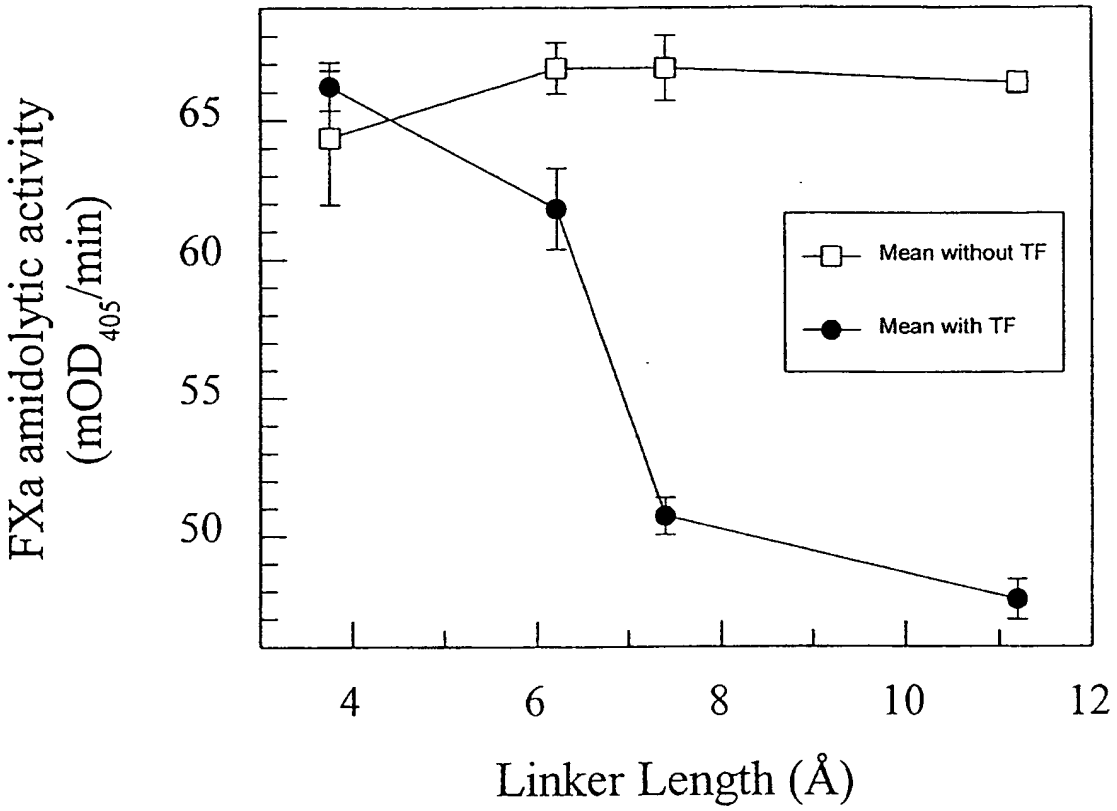

FXa amidolytic activity
(mOD405/min)
65
60
55
50
4
6
8
10
12
Linker Length (Å)
Mean without TF
Mean with TF

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00516

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07K 14/81, A61K 38/55
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5242810 A (JOHN M. MARAGANORE ET AL), 7 Sept 1993 (07.09.93) --	1-24
A	WO 9961055 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY), 2 December 1999 (02.12.99) --	1-24
A	US 5106833 A (GEORGE J. BROZE, JR. ET AL), 21 April 1992 (21.04.92) -- --------	1-24

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 January 2001	21.02.01
Name and mailing address of the ISA/ European Patent Office Facsimile No.	Authorized officer CAROLINA GÓMEZ LAGERLÖF/EÖ Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00516

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 23-24
because they relate to subject matter not required to be searched by this Authority, namely:
see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Claims 23-24 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

27/12/00

International application No.
PCT/DK 00/00516

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5242810 A	07/09/93	AU 9141791 A	08/07/92
		EP 0560913 A	22/09/93
		JP 6503474 T	21/04/94
		WO 9210575 A	25/06/92
WO 9961055 A1	02/12/99	AU 4093799 A	13/12/99
US 5106833 A	21/04/92	AU 604138 B	06/12/90
		AU 1928688 A	02/02/89
		BR 1100464 A	08/02/00
		DK 413588 A	24/01/89
		EP 0300988 A	25/01/89
		FI 97298 B,C	15/08/96
		FI 883477 A	24/01/89
		IL 87172 A	29/12/94
		JP 1047799 A	22/02/89
		JP 2781391 B	30/07/98
		KR 9007261 B	06/10/90
		NO 174776 B,C	28/03/94
		NO 883271 D	00/00/00
		NZ 225534 A	26/02/91
		PT 88075 A,B	31/07/89
		US 4966852 A	30/10/90
		US 5466783 A	14/11/95
		US 5773251 A	30/06/98
		US 5849875 A	15/12/98
		ZA 8805370 A	25/10/89
		ZA 8805371 A	26/04/89
		AT 111518 T	15/09/94
		AU 604139 B	06/12/90
		AU 1928788 A	25/05/89
		DE 3851511 D,T	30/03/95
		DK 413488 A	24/05/89
		EP 0318451 A,B	31/05/89
		ES 2063769 T	16/01/95
		FI 93365 B,C	15/12/94
		FI 883487 A	24/05/89
		IE 65393 B	18/10/95
		IL 87171 A	31/08/95
		JP 1165383 A	29/06/89
		JP 2836823 B	14/12/98
		JP 11075875 A	23/03/99
		KR 9300274 B	14/01/93
		NO 883270 A	24/05/89
		NZ 225535 A	27/11/90
		PT 88076 A,B	01/08/88